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CONTENT

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GC-MS Analysis of phytochemical compounds from Javan olive leaves (Olea javanica) extract and bioactivity screening of antidiabetes through in silico approach

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JTL 13 (1) JUNE 2022
Page: 15-24

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Original Research

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Lansium domesticum: A Natural Dual-Action Defense Against Dengue Mosquitoes

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Abstract: Preventing dengue virus transmission, which leads to Dengue Hemorrhagic Fever (DHF), can be effectively achieved using plant-based insecticides. This study investigates the potential of *Lansium domesticum* extract as both a repellent and a larvicide against *Aedes aegypti*, the primary vector of dengue fever. Five concentrations of *Lansium domesticum* extract (10%, 20%, 30%, 40%, and 50%) were tested for their repellent properties, while larvicidal efficacy was evaluated at concentrations of 20%, 40%, 60%, 80%, and 100%. The evaluation of larval mortality obtained an average larval mortality of 5%-20%, then a statistical analysis was carried out using the Anova test. Furthermore, probit analysis was carried out to determine the LC50. Results showed that all concentrations had a repellency rate greater than 50%. The larvicidal tests indicated that the 100% concentration was the most effective in killing larvae. These findings highlight *Lansium domesticum* as an effective natural repellent and larvicide against *Aedes aegypti*, offering a promising plant-based solution for dengue fever prevention.

Keywords: *Lansium domesticum*; reppelant; larvicidal

INTRODUCTION

Dengue hemorrhagic fever (DHF) is caused by the dengue virus, transmitted by the anthropophilic Aedes mosquito. These mosquitoes often live close to humans and are commonly found indoors. Asia ranks first globally in dengue cases, with a significant number of sufferers each year.[1] Current vector control methods primarily involve chemical means, such as fogging, which has been reported to be less effective in killing targets and increasing vector resistance to insecticides. [2,3,4,5]

The eradication of dengue vector mosquito larvae has traditionally involved using Temephos. However, Temephos usage raises significant environmental pollution concerns. Similarly, the widespread use of DEET (N, N-Diethyl-meta-toluamide) as an insect repellent presents several issues, including skin toxicity and potential impacts on the central nervous system if not used properly.[6] The chemicals used in synthesizing repellents often contain halogenated hydrocarbons, which have a long half-life and are known for their toxic properties.[7,8] These insecticides also leave toxic residues in food, water, air, and soil, leading to the resurgence and resistance of insect pests and negatively affecting non-target organisms. Over 645 species of insects and mites have developed resistance to insecticides, with 542 species of arthropods resistant to at least one compound. Approximately 7,470 cases of resistance

have been reported in insects against specific insecticides, with 16 arthropod species accounting for 43% of these cases.[9]

Dengue virus transmission can be prevented using plant-derived insecticides, which can target adult mosquitoes, larvae, and serve as protection against mosquito bites (repellents). Larvicides are substances designed to kill larvae, while repellents work locally or at a distance to prevent mosquitoes from flying, landing, or biting the skin of humans and animals. Using repellents can significantly reduce exposure to mosquito bites that may carry the dengue virus.[10]

Considering the negative effects of chemical larvicides and synthetic repellents, it is essential to study and find natural alternatives. The *Lansium domesticum* shows potential as a natural insecticide.[11] This is based on empirical evidence that rural communities in Southeast Sulawesi have long used langsat skin to repel mosquitoes. Studies have also reported that langsat skin can be used as an electric mosquito repellent.[12,13]*Lansium domesticum,* commonly referred to as langsat, is a medicinal plant extensively cultivated in Southeast Asia.[14] Capable of reaching heights of up to 30 meters, this plant has yielded several bioactive compounds,[11,15,16,17,18] some of which exhibit potential as antimalarial,[19] antibacterial,[20] antifeedant,[14] antimutagenic,[21] and insecticidal agents.[22] Previous research has identified alkaloids, flavonoids, saponins, triterpenoids, and tannins in the bark of *Lansium domesticum* stems, demonstrating larvicidal properties. In addition, studies conducted in Indonesia have shown the potential of plants that contain several bioactive compounds such as flavanoids, saponins, alkaloids found in *Citrus lemon* and *Pogostemon cablin* that show larvicidal and repellent activity.[23,24] Given the variability in secondary metabolite levels among different langsat plant varieties, it is imperative to investigate the specific variety found in Southeast Sulawesi. The analysis of secondary metabolites will provide valuable insights into its efficacy as a repellent and larvicide, offering a natural and sustainable alternative to chemical insecticides.

MATERIAL AND METHOD

This study is experimental study with post-test only control group design. The research procedure starts with collecting samples of the bark and stems of *Lansium domesticum* from Konawe Regency, Southeast Sulawesi. The bark and stems are then washed, dried, and ground into powder, followed by phytochemical screening preparations.

The bark and steams of *Lansium domesticum* are washed with running water, drained, and chopped. Samples are dried in the open air, protected from sun exposure. The dried samples are then ground into a powder and placed in a beaker with ethanol solvent at a 1:4 ratio. The samples are soaked for 5 days, occasionally stirred for homogenization. The liquid extract is filtered using filter paper, and the filtrate is collected. The sample is then macerated for 2 days with fresh ethanol solvent. The macerated sample is filtered, and the filtrate is combined with the previously collected filtrate. The combined filtrate is concentrated using a rotary vacuum evaporator to remove the solvent. This procedure is also used for the bark extraction of *Lansium domesticum*.

Procedure for Making Repellent Lotion

The lotion uses *Lansium domesticum* peel extract and involves weighing all ingredients and preparing a mortar. Ingredients include cetyl alcohol, stearic acid, methyl paraben, Adeps lanae TEA, glycerin, olive oil, and aquadest. The water phase (methyl paraben, warm water, TEA, glycerin) and oil phase (stearic acid, cetyl alcohol, lanolin, propyl paraben, patchouli extract) are heated separately at 70°C until homogeneous, then combined and stirred to form a lotion. Lotions are made at concentrations of 10%, 20%, 30%, 40%, and 50%.

Repellent Testing Against *Aedes aegypti*

The method using Arm-in-Cage Test. This method is commonly used to evaluate the effectiveness of mosquito repellents. In this procedure, a human volunteer (probandus) places an arm treated with repellent and an untreated arm into a container with mosquitoes to observe and compare the number of landings or bites on each arm. The process of testing *repellant* against *Aedes aegypti* follows: (1) Mosquitoes are included in the test container. Each container put 25 female mosquitoes; (2) *Repellent* lotion preparation is applied to the left arm and the right hand is not smeared. This presentation was carried out for 5 minutes; (3) Furthermore, the number of mosquitoes that landed during the exposure was calculated, both on the test arm and the control arm; (4) The test is carried out for 6 series, where each test series is carried out for 35 minutes and exposure is 5 minutes.[26] During the *repellent*, probandus was unable to wipe or wash hands. The protection or repulsion power can be known how the level of effectiveness after calculated based on the formula of Schreck et al, namely: % (repellency) = [(*Ta- Tb*)/*Ta*] × 100, where, Ta is the number of mosquitoes in the control and Tb is the number of mosquitoes on the test treatment.[27]

Larvicide Test

The larvicidal test uses *Lansium domesticum* stem extract. Twenty-five *A. aegypti* instar III larvae are placed in a test bottle containing the extract, Temephos (positive control), or aquadest (negative control). Larvicidal activity is observed for 24 hours. The mortality rate of the test larvae due to the larvicidal extract is recorded.

Data Analysis

The average larval mortality rate is statistically analyzed using ANOVA. Probit analysis is then conducted to determine the LC50 value.[16]

RESULTS AND DISCUSSION

The results of phytochemical screening of Lansium domesticum peel and stem compound can be seen in table 1.

Table 1. Test results of secondary metabolites content of Lansium domesticum

No	Parameters	Lansium domesticum peel	Lansium domesticum stem	Standar indicator
1	Alkaloid	+	-	An orange or red precipitate or a white precipitate is formed
2	Flavonoid	-	+	Changes color from green to orange or yellow
3	Saponin	-	+	Stable foam is formed
4	Triterpenoid	+	+	Formed red or there is a brownish ring
5	Steroid	-	-	A blue-green color is formed
6	Tannin	+	-	Blackish brown and precipitate formed
7	Fenol	+	-	Changing green to black

Note:
(+) = Positive contains compound
(-) = Negative contains compound

The results of the phytochemical screening of *Lansium domesticum* show that the bark extract of *Lansium domesticum* contains Alkaloids, Triterpenoids, Tannins and Phenols. While the stem extract of *Lansium domesticum* contains flavonoids, saponins, and triterpenoids. The results of phytochemical screening were carried out to determine the chemical components of *Lansium domesticum* which were identified qualitatively.

The composition of the lotion based on Lansium domesticum peel extract and the results of the physical stability test can be seen in table 2 and table 3.

Table 2. Composition lotion of *Lansium domesticum*

No	ingredient	Function	concentration				
			10 %	20%)	30%	40%	50%
1	Extract of Lansium domesticum	Active substance	10 %	20 %	30 %	40 %	50%
2	Cetyl alkohol	moisturizer	3 %	3 %	3 %	3 %	3 %
3	Asam stearat	emulsifier	10 %	10 %	10%	10%	10%
4	Metyl parabean	Preservative	0,15%	0,15%	0,15%	0,15 %	0,15 %
5	Adeps lanae	Addition substance	2 %	2 %	2%	2%	2%
6	TEA	Humektan	2 %	2%	2%	2%	2%
7	Gliserin	Humektan	10 %	10%	10%	10%	10%
8	Olive oil	solvent	10 %	10%	10%	10%	10%
9	Aquadest	solvent	100 %	100%	100%	100%	100%

Table 2 shows the chemical composition for each concentration of Lansium domesticum skin is the same, namely Cetyl alcohol 3%, stearic acid 10%, Methyl parabean 0.15%, adeps lanae 2%, TEA 2%, glycerin 10%, Olive oil 10% and aquadest 100%.

Table 3. Results of homogenity test and organoleptic lotion *Lansium domesticum*

Concentration reppelant	Homogenity test	Organoleptis test
10 %	Viscous form, light brown color and aromatic characteristic odor	Homogeneous, without air bubbles
20 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles
30 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles
40 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles
50 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles

Table 3 shows that all concentrations of Lansium domesticum skin reppelant lotion showed the results of the homogeneity test which were thick, light brown in color and characteristically aromatic. The organoleptic test results showed a homogeneous lotion consistency and no air bubbles.

Table 2. Test of Reppelant Lotion *Lansium domesticum* against *Aedes aegypti*

Note: C (+) = control positive
C (-) = control negative

No	Formulation concentration of Lansium domesticum	Total of masquitoes	Protective power (%)						
			0	1	2	3	4	5	6
1	10 %	25	91%	89%	90 %	89%	88%	87%	85%
2	20 %	25	92%	91%	90%	90%	87%	85%	85%
3	30 %	25	92%	89%	88%	89%	88%	87%	85%
4	40 %	25	92%	91%	88%	87%	87%	85%	85%
5	50 %	25	92%	89%	85%	82%	79%	76%	75%
6	C(+)	25	100%	100%	100%	98%	95%	94%	94%
7	C(-)	25	0	0	0	0	0	0	0

Table 3 shows that the protective power of Lansium domesticum peel extract for all concentrations is quite effective because it has a repulsion power above 50%. The effectiveness of langsat fruit peel extract in producing a repulsion of more than 50% against Aedes aegypti is due to the active substance content of the langsat fruit peel. Research has shown that the higher the concentration of *repellent*, the higher the repellency of mosquitoes.[24] Furthermore, in another study, it was also stated that plants that had the highest repellent potential wereplants from the family *Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae,* and *Rutaceae*.[28] plant *Lansium domesticum* from the Meliaceae family. The main volatile oil components of plants with *repellent* are *monoterpenoids* such as *geraniol, citronellol, linalool, terpineol*, *thymol, q-cymene, -bulnesene, patchouli alcohol, -pinene, -patchoulene*and *carvone.*[26,29,30,31]

From the Reppelency test, it can be seen that in the first hour the percentage of repulsion increases and then continues to decrease until the 6th hour. sixth. This is in accordance with the research of Pebrianti, et al.[32]

Larvicide test of Lansium domesticum on the mortality of Aedes aegypti

The results of the larvacide test of Lansium domesticum stem extract can be seen in Figure 1 below.

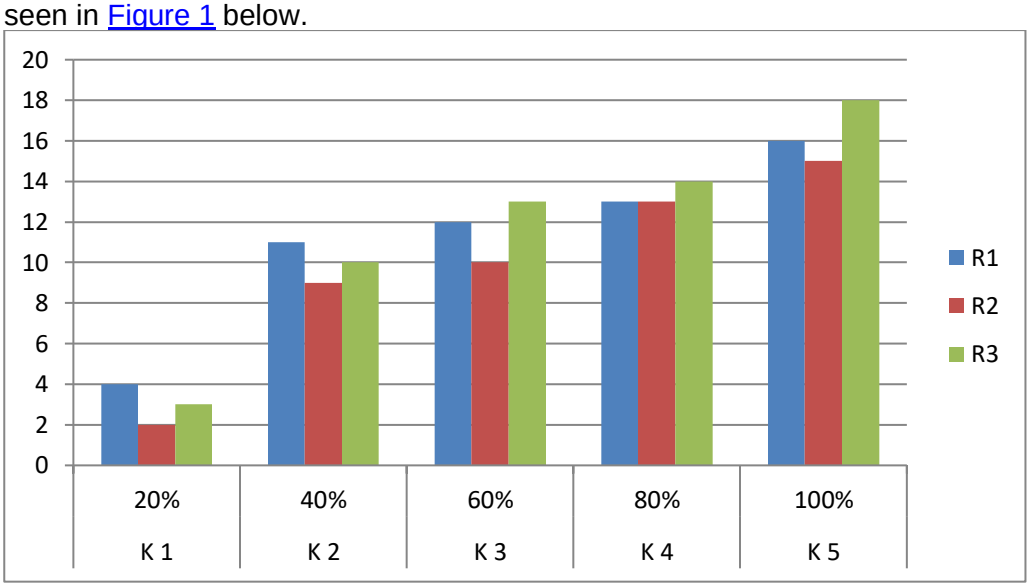

Figure 1. Larvicide test results of langsat bark on Aedes aegypti mortality

These results indicate that the highest percentage of *Aedes aegypti* was found in Lansium domesticum stem extract with a concentration of 100%, while the smallest larval mortality percentage was found in langsat stem extract at 20%.

Analysis of ANOVA test and Probit test

Data analysis to determine mortality mortality of Aedes aegypti larvae is using ANOVA test, which can be seen in table 4 below.

Table 4. Results of statistical tests using the Anova

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	900,952	6	150,159	150,159	,000
Within Groups	14,000	14	1,000		
Total	914,952	20			

The table of Anova test results shows the calculated F value of 150,159 which is greater than the good F table at the 1% significance level of 2.85 and at the 5% significance level of 4.46. This means that there is a difference in the average mortality of mosquito larvae at each concentration of langsat skin infusion. The significance value shows 0.000 is smaller than 0.05 which means that each concentration of langsat skin infusion has an effect on the mortality of *Aedes aegypti*.

In the study, the 50% Probit analysis test was carried out which can be seen in the following table.

Table 5. Probit analysis of larvicidal power (LC 50)

Larvicidal Power (LC)	24-hour time (%)	Range limit	
		Below	Upper
LC50	47.571	10,001	85.286

Based on the table above, it shows that the LC50 value is 47.51, which means that the concentration required for the death of Aedes aegypti larvae by 50% is 47.571%. The bark of langsat used in this study is the bark of old langsat which is still fresh, characterized by the bark of the stem being greenish brown or grayish, cracked and white. Observations of larval mortality showed that the percentage of larval mortality always increased with the high concentration used, this is in line with the theory that the higher the concentration of a larvicide, the higher the number of larval deaths, and the longer the exposure time, the higher the mortality of larvae. Alkaloids, terpenoids and flavonoids are plant defense compounds that can inhibit the eating process of insects and are also tox.

Flavonoids and saponins themselves function as respiratory tract inhibitors. Flavonoids function as respiratory inhibitors, where flavonoids function to disrupt energy metabolism in the mitochondria by inhibiting the electron transport system so that obstacles in the electron transport system will block ATP production and cause a decrease in oxygen use by mitochondria and cause larvae to have difficulty breathing. [33]

Triterphenoid compounds are one of the secondary metabolites that are found in large quantities and various molecular frameworks. Terpenoids are plant components that have an odor and can be isolated from plants by distillation called essential oils.[34] This compound is a repellent (Reppelant) because it has an odor that insects do not like. These compounds will enter through the respiratory tract through the food eaten by insects and these substances are absorbed by the digestive tract.

The difference in the mortality of mosquito larvae at each concentration is due to the difference in the sensitivity of each larva to the concentration of langsat bark, where the higher the concentration used, the higher the level of viscosity and concentration of the langsat stem extract, so that the movement space of the larvae is not as limited

as that of the larvae. being in the natural environment or in the outside environment, causing the larvae to have difficulty breathing and taking air on the surface of the water which results in insufficient oxygen for the larvae to grow, causing the death of the larvae.[35] The results of the probit analysis showed results that were in line with Handito's [36] study which reported that the greater the concentration, the greater the toxicity of a solution to Aedes larvae, so that the number of mosquito larvae mortality also increased. This happens because the more toxic compounds that enter the larva's digestive tract, they will bind to the receptors on the digestive cell membrane, cause damage to the cell membrane that leads mortality of larvae.

CONCLUSION

Lansium domesticum from the Meliaceae family that has the potential as a repellant and larvacide against *Aedes aegypti* → *perbaiki lagi, tambhkan implikasi dari hasil penelitian ini apa.*

AUTHORS' CONTRIBUTIONS

Reni Yunus: Corresponding authors, prepared the samples, designed the protocols, executed the protocols, wrote the manuscript, submit and revision and review the manuscript. Anita Rosanty: collection. data analytic and visualization statistically. All authors have read and approved the final manuscript

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DATA AVAILABILITY STATEMENT

The utilized data to contribute in this research are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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JTL 13 (1) JUNE 2024
Page: 25-32

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Post-vaccination SARS-COV-2 IgG Level: An evaluation study on 2 Area In Indonesia

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Abstract: Corona Virus Disease (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has become a global pandemic. Currently there is no effective treatment for this disease, vaccination is one way to deal with this disease. Antibodies that are formed after vaccination are expected to provide protection for everyone. This study was to determine the levels of IgG antibodies formed after SARS-CoV-2 vaccination. 87 respondents were examined in this study using blood samples. Measurement of antibody levels using the CLIA method. The results obtained show that the average level of antibodies formed is 193.355 BAU/ml. 65 respondents who received 3 doses of vaccine (199.652 BAU/ml) had higher antibody levels than respondents who received two doses of vaccine (175.531 BAU/ml) and 1 dose of vaccine (158.365 BAU/ml). Antibody levels in respondents who were examined between 0-6 months after vaccination (202.827 BAU/ml) had higher levels than respondents with a period of more than six months (186.010 BAU/ml). From the study results, data was obtained that the antibody levels in recipients of three vaccine doses were higher so that they could provide sustainable protection against COVID-19. Therefore, the COVID-19 vaccine booster should give to all people to provide protection against COVID-19.

Keywords: *SARS-CoV-2; Vaccination; IgG COVID-19.*

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INTRODUCTION

Corona Virus Disease (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been a global pandemic since March 2020. COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a single-stranded RNA Betacoronavirus, with no effective treatment available. Vaccination campaigns worldwide aim to reduce COVID-19 morbidity and mortality. The vaccination program In Indonesia, aims to reduce the number of suffering and deaths due to COVID-19 achieve group immunity in society (herd immunity) [1,2,3]. Several types of vaccines used are live attenuated vaccines, inactivated vaccines, protein subunit based vaccines, virus-vector based vaccines, and nucleic acid based vaccines [4]. Additionally, large-scale social restrictions have been implemented to limit movement and prevent transmission [5]. Ensure all facts are accurate and clearly presented. For example: "By 2020, over 30 vaccines had undergone clinical testing, using various platforms: inactivated virus (e.g., Sinovac, Sinopharm), mRNA (e.g., Moderna, BioNTech/Pfizer), protein subunits (e.g., Novavax), and viral vectors (e.g., AstraZeneca/Oxford, CanSino Bio, Johnson & Johnson, Gamaleya) [6,7]. Each type has distinct advantages and disadvantages. Inactivated virus vaccines, being the safest, use killed pathogens and pose no risk of infection, though they elicit a relatively low immune response. mRNA vaccines use genetic sequences but require specific temperature conditions for storage. Protein subunit vaccines use viral proteins or segments to induce strong humoral and cellular immunity. Viral vector vaccines can be produced on a large scale, but pre-existing immunity can reduce their effectiveness [5,7,8,9,10,11].

Vaccination-induced protection is mediated through complex interactions between innate, humoral, and cellular immunity. Among those immune responses, the humoral response is much easier to detect than others because of tis wide use and standardization[12,13]. Various tests have been developed to detect immunoglobulin M (IgM), IgA, and IgG antibodies from blood samples of patients who have been or are currently infected with COVID-19. This serological test is carried out using various viral antigens and recombinant proteins to capture specific antibodies for SARS-CoV-2 [14,15].

The Spike protein (S) expressed by SARS-CoV-2, nucleocapsid protein (NCP), and other structural proteins are known to be the main targets of antibodies. NCP is related to the viral genome, in the early stages of infection this protein is produced in large quantities. NCP's high sensitivity means no cross-reactivity even with closely related viruses. Therefore, the antibody test for NCP is quite specific[16,17]. The S-protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface. Protein S consists of two subunits, S1 and S2. Viral entry into the cell is mediated by a receptor binding protein (RBD) within the structure of the S protein. Since the S protein has an important role in the viral entry into cells, it is an important target in virus inactivation and the post-vaccine immune response[18]. Both of these proteins are highly immunogenic and are used as essential proteins in testing for COVID-19 [19].

After vaccination the antibody response that is formed varies depending on the type of vaccine injected. Antibody response to vaccines with inactivated viruses occurs within 14-21 days. For mRNA vaccines this occurs within 21-28 days. In the protein subunit vaccine it occurs within 21 days and in the vector virus vaccine it occurs in 28 days. Demographic characteristics gender, age, and body mass index may have an important role in the development of the immune response after vaccination[20]. The presence of co-morbidities can also affect the immune response after vaccination [21].

Antibody response to COVID-19 vaccination is considered very important for protection from this disease. In general, the antibody response to vaccination may differ depending on the population studies[22]. Antibody towards COVID-19 vaccination was higher in females compared to males as initial response up

to several weeks[23],positivity decreases with age, and positivity is lower in transplant recipients, obese individuals, smokers and those with specific comorbidities [24].

In this study we examined the levels of IgG formed against S-RBD after SARS-CoV-2 vaccination. IgG plays an important role in defending against SARS COV2 infection, including antibodies to the receptor-binding domain (RBD) of the SP, which strongly correlate with antibodies that neutralize viral replication, and play in controlling infection and in disease pathogenesis[25].

MATERIAL AND METHOD

This study is a descriptive analysis aimed at evaluating the levels of IgG antibodies formed after SARS-CoV-2 vaccination. Samples were collected from individuals who had received the SARS-CoV-2 vaccination in Denpasar, Bali, and Cimahi Regency, West Java. This research was conducted from January 2021 to December 2021. The sample size was determined using a simple random sampling technique from the population of vaccinated residents in Denpasar and Cimahi. The unit of analysis was the serum of vaccinated residents

The sampling procedure began with preparing the necessary materials and tools and using complete personal protective equipment (PPE), followed by collecting a blood sample. Samples were centrifuged for 15 minutes at 2000-3000 RPM at 2-8°C [26].

The sample examination procedure begins with the preparation of reagents. Reagents were removed from the box and checked to ensure they were in good condition, by ensuring not passed the expiry date, and still in properly sealed packaging. The reagent barcodes were scanned into the CLIA (Chemiluminescence Immuno Assay) tool system to detect the LOT number. Magnetic microbeads were resuspended and homogenized thoroughly. Perform calibration by clicking the calibration button to run the calibration operation. Then a sample test is carried out by placing the patient's serum in the "Sample Area" and clicking the button to run the test [27].

The data obtained both primary and secondary data are recorded, collected, processed, and presented in the form of narratives and tables.

RESULTS AND DISCUSSION

In this study there were 87 respondents. Of the 87 respondents, 80% of all respondents were women. All respondents had received the first, 86 respondents had received second doses of the vaccine, and only 65 respondents had received the third dose of the vaccine. The mean IgG antibody level formed was 193.355 BAU/ml. A total of 33 respondents received the same type of vaccine while 54 others received a different type of vaccine during vaccination. Respondent characteristics can be seen in the **Table 1**.

Table 1. Characteristics of Respondents

Characteristics of Respondents	Number of Respondents	%
Gender		
Men	17	19.54
Women	70	80.46
Vaccine Dosage		
First Dose	87	100
Second Dose	86	98.85
Third Dose	65	74.71

Long After Vaccination		
0-6 months	38	43.68
>6 months	49	56.32
The Type of Vaccine		
First Dose	87	100
Sinovac/Coronavac	63	72.41
Astra Zeneca	20	22.99
Pfizer	3	3.45
Moderna	1	1.15
Second Dose	86	100
Sinovac/Coronavac	63	73.26
Astra Zeneca	19	22.09
Pfizer	3	3.49
Moderna	1	1.16
Third Dose	65	100
Sinovac/Coronavac	3	4.62
Astra Zeneca	26	40
Pfizer	25	38.46
Moderna	11	16.92
Antibody Levels (BAU/ml)		
Minimum	12.422	
Maximum	394.029	
Mean	193.355	

The mean of IgG antibody levels in 65 respondents who received three doses of vaccine (199.652 BAU/ml) was higher than the respondents who received one (158.365 BAU/ml) and two doses of vaccine (175.531 BAUml). IgG antibody levels formed between 0-6 months (202.827 BAU/ml) after vaccination until the examination was carried out were higher than antibody levels formed after more than six months (186.010 BAU/ml).

Table 2. The Mean of IgG Antibody Levels

	Number of Respondents	SARS-CoV-2 IgG S-RBD Antibody Levels (BAU/ml)
Number of Vaccines		
1 time vaccine	1	158.365
2 times vaccine	86	175.531
3 times vaccine	65	199.652
Vaccine Time		
0-6 months	30	202.827
>6 months	49	186.010

After the COVID-19 vaccination the antibody response that is formed is important for a person's immunity. Vaccination is a well-known and powerful humanitarian weapon in the fight against COVID-19. Population immunity triggered by rapid vaccination is an important global strategy for controlling COVID-19. Vaccination programs must maximize initial effect so as not to face the faster spread of new variants[28]. Administration combination/heterologous vaccines (primer and booster) schedule or prolonged vaccination interval induces robust humoral immunogenicity with good tolerability. Extending the time to boost-immunization is key to both improving antibody induction and reducing ADR rate [29].

Antibodies are formed by exposure of the immune system to vaccine antigens thereby stimulating the formation of memory B cells, which differentiate

into antibody-secreting cells. IgG molecules are produced by antibody-secreting cells and are responsible for vaccine-induced immunity. The results of this study are in line with other studies which also found that antibody titers tended to increase significantly up to day 36 or the first month after vaccination[30]. A similar study conducted by Trougakos et al. (2021) which assessed the kinetics of antibody response after administration of BNT162b2 mRNA vaccination, found that there was a sharp increase in anti-IgG S-RBD levels in vaccinated recipients after day 22 and remained high at day 50 [31].

Terpos et al. (2021) revealed that the decrease in antibodies formed after vaccination occurred on day 36 to day 111. However, the antibody response persisted in the body on day 111. This indicates that there is ongoing immune protection against COVID-19 [30]. This is in line with the results of our study. The results of our study showed that respondents with a vaccine duration of more than six months had lower IgG antibody levels compared to respondents with an examination period of 0-6 months after vaccination. Other study also stated that the efficacy of the mRNA vaccine for SARS-CoV-2 for up to six months has an efficacy of up to 73% in fully vaccinated individuals. The effectiveness rate of the vaccine in first 5 months reaches 90% and decreases to 47% after 5 months. Tartof et al. (2021) suggest that additional doses may be required up to six months after two doses of the vaccine to increase its efficacy [32].

Differences in antibody levels that are formed can also be caused by the type of vaccine. Larkin (2022) stated that different types of SARS-CoV-2 vaccines can provide different protections. This can be used as a strategy to guide coordinating vaccine variants to increase protection against new variants and can have implications for the development of future therapies [33]. Besides that, the intrinsic factors of the respondents also influence the immune response that is formed after vaccination. These factors include age, sex, genetics, and comorbidities [12]. A study showed that age has a significant effect on antibody levels after vaccination. It was found that individuals at a younger age (<50 years) maintained higher antibody levels after day 36 [30]. Other study has also consistently shown that there is an effect of age on the antibody response formed after vaccination with the mRNA-1273 vaccine [34]. Apart from the antibodies that are formed, the efficacy of the vaccine also effects the symptoms after a person is infected with COVID-19. A study conducted by Masrike et al. (2023) stated that a person who received two doses of the vaccine was reported to have a post-COVID-19 chronic cough with a lower frequency [35]. This showed that the booster of the SARS-CoV-2 vaccine is needed byb the community in tackling the COVID-19 pandemic.

Our study has several limitations. First, the number of samples was relatively small, indicate limited representativeness of results [36]. Second, the demographic skew towards women, which females were more prone to adverse reactions after vaccination. This can be influenced by hormonal factors (where the estrogen has a better immune response compared to the testosterone), as well as genetics (where the x chromosome has an immune response 10 times better than the y chromosome) [23]. Exploring the of antibody levels periodically and long-term period, vaccine combinations or the impact of booster doses in diverse populations, as well as a larger number of samples in diverse populations could be highlighted as important for the next study

CONCLUSION

The study found that the levels of IgG antibodies formed after COVID-19 vaccination increased significantly within the first 0-6 months and decreased thereafter. Specifically, the mean antibody levels were higher in respondents who had been vaccinated within the last six months (202.827 BAU/ml) compared to those vaccinated more than six months ago (186.010 BAU/ml). Additionally, respondents who received three doses of the vaccine had higher antibody levels (199.652 BAU/ml) than those

who received only one (158.365 BAU/ml) or two doses (175.531 BAU/ml). These findings underscore the necessity of booster vaccines to maintain elevated antibody levels and ensure sustained protection against COVID-19. Furthermore, the data indicates that using a mix and match strategy with different vaccine types could enhance immune protection. The majority of respondents received different vaccines for their subsequent doses, suggesting that heterologous vaccination regimens might be effective in eliciting robust antibody responses. This approach could be strategically employed to enhance immunity, particularly in light of emerging variants and the waning of antibody levels over time. Therefore, ongoing vaccination programs should consider incorporating booster doses and potentially varying vaccine types to optimize long-term immunity against COVID-19.

AUTHORS' CONTRIBUTIONS

Bekti: Conceptualization; Methodology; Investigation. Dharmawati: Data curation; Project Administration. Habibah: Visualization; Writing-Original Draft. Merdekawati: Resources; Investigation. Noviar: Resources; Investigation. Suiraoka: Methodology; Data curation. Rinawati: Resources; Writing-Original Draft. Syahniar: Writing-reviewing and editing. Ayatullah: Visualization

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigastion are available from the corresponding author on reasonable request

DISCLOSURE STATEMENT

There is no conflict of interest.

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Case Report

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Analysis polypharmacy in elderly patients with decreased kidney and liver function: A case report

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Abstract: Elderly patients are at a high risk of developing chronic diseases, including cardiovascular disease, cancer, dementia, and diabetes. Additionally, many elderly individuals experience multimorbidity, having more than one chronic condition, contributing to the phenomenon of polypharmacy. This case report aims to analyze polypharmacy therapy in elderly patients with concurrent decreased kidney function and elevated liver enzymes. Polypharmacy in elderly patients with decreased kidney and liver function necessitates a comprehensive study to assess both its benefits and potential side effects. Monitoring should be implemented to detect any emergence of side effects or drug interactions resulting from the prescribed therapy. According to the 2023 Beers Criteria, elderly patients with reduced kidney function face an elevated risk of drug-related side effects, potentially leading to prolonged hospital stays and increased medical costs.

Keywords: Polypharmacy, Renal injury, Liver injury, Beers Criteria.

INTRODUCTION

Elderly patients are defined as individuals aged 60 years and older. In 2017, 9.8% of Southeast Asia's population fell within this demographic, a figure projected to rise to 13.7% and 20.3% by 2030 and 2050, respectively. Notably, Indonesia faces a significant challenge, having the highest number of individuals with limited financial security in old age compared to other low to middle-income countries.[1]

The elderly population is particularly vulnerable to chronic diseases, including cardiovascular disease, cancer, dementia, and diabetes.[2,3] Diabetes mellitus, among the most prevalent chronic conditions in the elderly, affects up to 135.6 million individuals aged 65 years or older worldwide.[4] Other studies indicate that as age increases, the occurrence of comorbidities rises, with patients often experiencing more than one chronic disease.[5,6] This contributes to an upsurge in disease prevalence and a heightened susceptibility to receiving multiple medications, leading to polypharmacy.[7]

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The utilization of five or more medications amplifies the risk of drug side effects and drug-drug interactions.[8] Observational retrospective research demonstrates that polypharmacy can result in one in two elderly individuals experiencing potentially inappropriate medication (PIM), and the complexity of the regimen may lead to hospitalization.[7]

The profound alterations in body composition and the gradual decline in various systems and organs, including changes in renal and liver function, significantly impact the pharmacodynamics and pharmacokinetics of drugs.[2,9] Consequently, optimizing drug regimens becomes imperative in such cases. Several studies advocate the use of PIM assessment instruments, such as the Beers Criteria developed in the United States, to assist healthcare professionals in identifying PIM in elderly patients with specific clinical conditions. Another widely employed instrument is the STOPP/START criteria in Ireland.[10-13]

Decreased kidney function leads to drug and active metabolite accumulation, potentially triggering toxicity. Given the crucial roles of the kidneys, careful attention to drug selection and dose adjustment is essential to ensure the safety of drug use.[14] Dose adjustments in patients with renal failure aim to achieve optimal therapy, preventing undue stress on the kidneys due to elevated drug levels in the plasma.[15,16]

The liver, being the primary site for metabolism, plays a pivotal role in drug inactivation, converting substances through enzymatic processes into inactive metabolites or water-soluble forms for excretion. Some drugs undergo transformation into active metabolites, resulting in an augmented pharmacological response. Liver diseases, such as cirrhosis and hepatitis, significantly impact drug metabolism.[17] Patients with decreased liver function experience elevated drug levels in the plasma, particularly for drugs metabolized by the liver. Consequently, the dosage regimen for specific drugs must be adjusted based on the metabolic rate of individuals with impaired liver function.[18] This case report aims to analyze polypharmacy therapy in elderly patients with concurrent decreased kidney function and elevated liver enzymes.

CASE REPORT

Patient Mrs. P, 74 years old, presented with complaints of abdominal pain, nausea, and vomiting. She was admitted with a diagnosis of type-2 diabetes mellitus, high fat low hemicellulose (HFLH), and dyspepsia, with a history of metformin therapy. In the emergency room, her vital signs were recorded, revealing a blood pressure of 147/88 mmHg, oxygen saturation of 91%, and a pulse rate of 70 beats per minute, along with symptoms of nausea and vomiting. Throughout treatment, the patient reported weakness, with a Glasgow Coma Scale (GCS) of 456. Her laboratory examination results indicated a hemoglobin level of 11.3 g/dL, a leukocyte count of 13,700/µL, and a platelet count of 471,000/µL. Electrolyte values included sodium at 133 mEq/L, potassium at 4.25 mEq/L, chloride at 99.8 mEq/L, and calcium at 9.1 mg/dL. A decline in kidney function was evident with a Blood Urea Nitrogen (BUN) value of 31.1 mg/dL and serum creatinine of 6.66 mg/dL. Liver function assessment revealed elevated Serum Glutamic Oxaloacetic Transaminase (SGOT) at 192 U/L and Serum Glutamic Pyruvic Transaminase (SGPT) at 430 U/L, exceeding five times the normal limit. The patient's Glycated Hemoglobin (HbA1c) was 323 mg/dL. Chest thorax examination revealed cardiomegaly consistent with hypertensive heart disease (HHD) and pulmonary edema. Abdominal ultrasound (BOF) did not detect ileus or pneumoperitoneum.

During hospitalization, the patient received ondansetron injection therapy (8 mg every 12 hours), furosemide injection (20 mg), omeprazole injection (2x40 mg), and multivitamin injection (40 mg every 12 hours). Insulin therapy included Lantus at a dose of 24 units at night and insulin glulisine at a dose of 8 units every 8 hours. The patient was also administered domperidone (10 mg every 8 hours),

spironolactone (25 mg every 24 hours), and candesartan (4 mg every 24 hours). After five days of treatment, the patient's blood sugar levels consistently decreased, reaching the therapeutic target of below 180 mg/dL on the 4th day with the insulin combination. Following six days of treatment, the patient was discharged with improved nausea and vomiting complaints and a random blood sugar level of 143 mg/dL. Notably, no drug interactions were identified in this case.

RESULTS AND DISCUSSION

The examination of kidney and liver function results reveals a decline in kidney function and alterations in liver function. A longitudinal study involving 916,619 elderly patients over 65 years old demonstrated that alterations in kidney and liver function were correlated with multimorbidity. Renal function changes were observed in patients with cardio-renal disorders (OR 2.19; 95% CI 2.15–2.23) and those with metabolic diseases (OR 2.16; 95% CI 2.12–2.20). On the other hand, impaired liver function was identified in patients with gastrointestinal disorders (OR 3.39; 95% CI 3.30–3.49) and cardio-renal conditions (OR 1.96; 95% CI 1.91–2.02).[19] Age-related changes in liver function exert a significant influence on drug clearance and variability in response to most drugs.[3] Consequently, ensuring appropriate therapy for patients is crucial to prevent the occurrence of potentially inappropriate medications.

During the patient's hospitalization, ondansetron and domperidone therapy were administered to address complaints of nausea and vomiting. Ondansetron is the preferred choice for elderly patients (>60 years) due to its absence of extrapyramidal syndrome side effects, unlike metoclopramide. According to the 2023 Beers Criteria, metoclopramide, an antiemetic, should be avoided (except in cases of gastroparesis lasting no more than 12 weeks) as it carries the risk of extrapyramidal syndrome, including tardive dyskinesia, which is more prevalent in the elderly population.[20] However, caution is required in the use of ondansetron, as it may cause QT prolongation occurring 1-2 hours post-administration, necessitating ECG monitoring.[21] Ondansetron does not require dose adjustment in patients with decreased renal function. In contrast, if liver function is compromised, ondansetron should be administered at a maximum daily dose of 8 mg.[22]

The patient was additionally prescribed domperidone as antiemetic therapy since complaints of nausea persisted. Domperidone facilitates peristalsis and gastric emptying by inhibiting dopamine D2 receptors in the gastrointestinal tract and various central and peripheral nervous systems. This prokinetic agent serves as a second-line therapy for gastroparesis in patients who have not responded to metoclopramide.[23]

It also increases esophageal and gastric motility, thereby expediting gastric emptying and elevating lower esophageal sphincter (LES) pressure, effectively alleviating symptoms of vomiting and regurgitation. Notably, domperidone stands out among prokinetic groups for its milder extrapyramidal effects.[24]

Metabolism of domperidone occurs during its passage through the liver and gastrointestinal tract, employing a first-pass elimination mechanism (first-pass metabolism); thereby, caution is warranted when administering this drug to patients with compromised liver function.[22] With high oral bioavailability, domperidone is absorbed orally and excreted through the kidneys, boasting a half-life (T1/2) of approximately 7-12 hours.[25] In individuals with decreased kidney function, the recommended dose of domperidone is 10–20 mg, administered 1-2 times per day.[22]

In addition to domperidone, the patient was prescribed omeprazole to address complaints of nausea and vomiting. According to the Beers Criteria, omeprazole use in geriatric patients may heighten the risk of *Clostridium difficile* infection, bone loss, and fractures.[20] The administration of proton pump inhibitors (PPIs) should

be approached with caution in geriatric patients, especially for prolonged therapy exceeding eight weeks. Careful consideration is also necessary when using omeprazole in patients with decreased liver function, as decreased drug metabolism processes may increase its bioavailability. Conversely, in patients with decreased kidney function, omeprazole can be administered at a standard dose without adjustment.[22]

To manage blood sugar levels, the patient received insulin hormone therapy. Insulin, synthesized by the β cells of the islets of Langerhans from proinsulin, plays a crucial role in stimulating the utilization and storage of amino acids, intracellular fatty acids, and glucose. It regulates blood glucose levels in the liver, muscles, and adipose tissues. In cases of hyperglycemia, reduced insulin levels impede the entry of blood glucose into muscle cells, adipose tissue, and the liver, disrupting metabolism.[26]

Insulin therapy for type-2 diabetes mellitus is initiated when oral therapy fails, blood glucose control is poor (A1c > 7.5%), or fasting blood glucose levels exceed 250 mg/dL. All insulin types are suitable for patients with reduced kidney function, and no specific insulin dosages are recommended. The selection of insulin type, dosage, and usage must be tailored to the patient's condition to achieve therapeutic goals without inducing hypoglycemia.[26]

Insulin glulisine, a rapid-acting insulin, is quickly absorbed in the body and can promptly reduce insulin levels for prandial insulin needs. It has an onset of action of 5-15 minutes, peak action at 30-90 minutes, and an average duration of 5 hours. In patients with stage 4-5 chronic kidney disease (CKD) or those undergoing dialysis, who often experience delayed gastric emptying, administering rapid-acting insulin helps synchronize insulin peaks with postprandial blood glucose peaks.[27]

For patients requiring constant basal insulin, long-acting insulin therapy is employed. The patient, in this case, received insulin glargine (Lantus). Long-acting insulin begins working within 1-2 hours, has almost no peak effect or evenly distributed peaks over 24 hours and maintains its effect for more than 24 hours. This insulin's advantage lies in its once-daily administration, typically at night, as it minimizes the risk of nocturnal hypoglycemia due to its lack of a pronounced peak effect.[28]

The patient is prescribed a combination of furosemide and spironolactone as diuretic therapy. However, the use of spironolactone with a GFR < 30 mL/min is not recommended according to the 2023 Beers Criteria due to the potential risk of hyperkalemia.[20] Spironolactone, an aldosterone antagonist, proves beneficial in cases of volume overload associated with heart problems, cirrhosis, and kidney disorders.[29]

Diuretics, particularly furosemide, play a crucial role in treating acute heart failure, characterized by excess fluid leading to peripheral edema. Diuretics effectively alleviate shortness of breath, enhancing the patient's capacity for physical activity. By reducing water and salt retention, diuretics diminish extracellular fluid volume, venous return, and preload. Typically, a potent diuretic like furosemide is administered at an initial dose of 40 mg, adjusted as needed to achieve sufficient diuresis. In geriatric patients, caution is essential to avoid volume depletion and hypotension, as baroreceptor function tends to decline. Typically, a potent diuretic like furosemide is administered at an initial dose of 40 mg, adjusted as needed to achieve sufficient diuresis.[30] In geriatric patients, caution is essential to avoid volume depletion and hypotension, as baroreceptor function tends to decline. Therefore, diuretics should be administered judiciously, especially in cases of asymptomatic heart failure or when there is no fluid overload.[31,32]

CONCLUSION

Polypharmacy in elderly patients with decreased kidney and liver function necessitates a comprehensive study to assess both its benefits and potential side effects. Vigilant monitoring of adverse effects in elderly patients is crucial.

AUTHORS' CONTRIBUTIONS

Rina Widiyawati and Khoirul Anam took research data and wrote this journal. Nur Oktafiyani, M. Hari Pristantiningtyas, Herya Putra Dharma and Muhammad Muchlis chose cases in the hospital that could be used as case reports, as well as guiding the writing of this journal. Jainuri Erik Pratama, Fauna Herawati, Adji Prayitno Setiadi and Marisca Evalina Gondokesumo reviewed and supervised the journal. All authors have read and approved the final journal.

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Case Report

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High dose glucocorticoids inducing hyperglycemia in patients with diabetes mellitus experiencingrecurent ischemic stroke attacks: A case report

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Abstract: Stroke is an acute and focal neurological syndrome characterized by clinical deficits resulting from vascular injuries such as infarction or hemorrhage in the central nervous system. Given the prevalence of multiple comorbidities among stroke patients, they often find themselves on more than five medications, falling into the category of polypharmacy. Beyond treatments aimed at improving stroke outcomes and managing comorbid conditions, the presence of additional diseases may necessitate new therapies, potentially leading to side effects that can intersect and exacerbate the existing disease. This case report aims to present instances of hyperglycemia in stroke patients undergoing high-dose glucocorticoid therapy and discuss potential strategies to address this issue. In this particular case, human insulin was selected to rapidly control the patient's hyperglycemic condition. Subsequently, adjustments to basal and bolus insulin doses were made based on the frequency of use and duration of action of the glucocorticoid, specifically methylprednisolone. In conclusion, the hyperglycemia induced by glucocorticoids was identified through postprandial sugar monitoring, necessitating treatment through modifications to basal and bolus insulin doses. Strategies for managing hyperglycemia should be tailored to the pharmacokinetics of glucocorticoids and insulin.

Keywords: Stroke, Polypharmacy, Comorbidities.

INTRODUCTION

Stroke is a sudden disturbance in brain function characterized by clinical signs and symptoms lasting more than 24 hours.[1] It results from either an ischemic or hemorrhagic process, often initiated by a lesion or injury to the arteries. Approximately two-thirds of strokes are ischemic, while one-third are hemorrhagic. Ischemic strokes are attributed to thromboembolic blockages in blood vessels, leading to an ischemic region beneath the obstruction. Conversely, hemorrhagic strokes result from the rupture of a microaneurysm.[2]

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In Indonesia, stroke accounts for 15.4% of deaths,[3] presenting the highest mortality incidence due to stroke in Southeast Asia—193.3/100,000 people per year—second only to Mongolia, with a mortality rate of 222.6/100,000 people per year.[4] The risk of recurrent ischemic stroke is notably elevated during the early post-acute phase, with at least 80% of ischemic events recurring in individuals with a history of ischemic stroke. A comprehensive preventive approach encompassing dietary modification, exercise, antiplatelet/anticoagulant therapy, antihypertensives, and statins is crucial.[5,6] However, this multifaceted treatment approach often leads to polypharmacy,[7]

defined as the prescription and use of more than five drugs. Analysis indicates that stroke patients with multimorbidity contribute to the escalating treatment burden.[8] The highest prescription rates are observed in patients with cardiovascular risk factors, particularly diabetes and coronary heart disease.[9] This comorbid therapy is essential for managing blood pressure, glucose levels, and lipid levels. Nevertheless, polypharmacy introduces the risk of drug interactions and side effects, potentially exacerbating the patient's condition.

Steroid therapy, particularly glucocorticoids, plays a pivotal role in treating and preventing acute and chronic inflammatory diseases, as well as disorders of the immune system.[10] The use of corticosteroids is intricately linked to various side effects, including fluid retention and edema, blurred vision, modulation of the immune response, and steroid-induced hyperglycemia. Other side effects encompass the development of avascular necrosis, cataracts, glaucoma, psychosis, impacts on the endocrine system, and the initiation of bone disease, dyslipidemia, obesity, and adrenal suppression.[11]

The prevalence of corticosteroid-induced hyperglycemia is contingent on the dose, indication, and context of use. Individual factors such as age, baseline body mass index (BMI), and a family history of diabetes are recognized as influencing the risk of developing steroid-induced hyperglycemia (SIHG).[12] Observational data indicates that approximately 2% of diabetes cases in the primary care population are attributed to corticosteroid therapy and contribute to the onset of new diabetes.[13] A meta-analysis revealed that in patients without a prior history of diabetes who received systemic glucocorticoids, the incidence rate of hyperglycemia was 32.3%, with 18.6% of this subgroup subsequently developing diabetes mellitus.[14] Conversely, in patients undergoing solid organ transplantation and glucocorticoid therapy, the prevalence of hyperglycemia ranged between 17% and 32%.[15,16] This case report aims to present instances of hyperglycemia in stroke patients undergoing high-dose glucocorticoid therapy and discuss potential strategies to address this issue.

CASE REPORT

Patient Mrs S, aged 52, presented with complaints of weakness on the right side of the body and difficulty swallowing. The patient had a history of cerebrovascular events one year prior and a concurrent diagnosis of diabetes mellitus (DM). The patient was on medication, although the specific details of the medication history were not recalled. The diagnosis upon admission was a second stroke accompanied by double hemiparesis. Additionally, the patient was diagnosed with bronchial asthma, sputum retention, and type 2 diabetes mellitus (DM).

Upon arrival at the emergency room, the patient's Glasgow Coma Scale (GCS) was 445, with a blood pressure of 215/116 mmHg, a pulse rate of 103 times/minute, and normal body temperature, respiratory rate, and O2 saturation. Her laboratory examination revealed a Hb value of 16.0 g/dL, a leukocyte count of 11,100 cells/mm^3, and a platelet count of 384,000 platelets/mm^3. The patient's serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) values were 28 U/L and 53 U/L, respectively. Her electrolyte levels were as follows: sodium 132.3 mmol/L, potassium 4.11 mmol/L, chloride

107.3 mmol/L, and calcium 4.44 mmol/L. The total cholesterol level was 252 mg/dL, with LDL at 139 mg/dL and HDL at 35 mg/dL. Hypertriglyceridemia was also noted, with a value of 542 mg/dL. A random blood sugar check in the emergency room showed a level of 248 mg/dL.

The emergency room treatment included IV Ranitidine 50 mg every 24 hours, IV Citicoline 500 mg every 24 hours, IV Ondansetron, SC Novorapid 3x10 units, and Levemir 18 units at night. Oral medications included Clopidogrel 1x1, Atorvastatin 20 mg 1x1, MgSO4 40% 5cc administered in 1 hour, N-acetylcysteine 3x200 mg, Cetirizine 10 mg, a combination of Salmeterol and Fluticasone, Methylprednisolone 62.5 mg every 12 hours, Nebule Combivent Budesma, and Lactulose 3x1c. On the 6th day of hospitalization, the patient received Actrapid 100 IU/50 cc NS. Following the administration of Actrapid, the blood glucose levels began to decrease from 534 to 300 at 7 hours after IV insulin. After nine days of treatment, the patient exhibited clinical improvement, manifested by an increase in GCS to 456.

RESULTS AND DISCUSSION

The recurrence of ischemic stroke poses a significant challenge due to the potential for broader physical and cognitive damage, leading to disability. Undetected risk factors contribute to the likelihood of recurrent strokes, as evidenced by a systematic review and meta-analysis highlighting large-artery atherosclerotic (LAA) and cardioembolic (CE) stroke patterns as common etiologies leading to recurrent events. This case report identified modifiable risk factors such as hypertension, diabetes mellitus, and atrial fibrillation as independent contributors to recurrence, aligning with the patient's condition of hypertensive emergency and uncontrolled blood sugar. Urgent implementation of secondary preventive therapy, including interventions to manage hypertension and hyperglycemia, becomes crucial to prevent similar events.[17]

During hospitalization, the patient received high doses of methylprednisolone for managing bronchial asthma. On the second day of post-methylprednisolone administration, a notable increase in the patient's blood sugar levels within the range of 358-534 mg/dL was observed. Glucocorticoids exhibit diabetogenic effects, particularly in individuals with insulin resistance, leading to hyperglycemia. The rise in sugar levels is attributed to increased postprandial blood sugar resulting from the metabolic impact of glucocorticoids.[18] This occurrence may be associated with the duration of action of glucocorticoids, classifying methylprednisolone as a medium-acting glucocorticoid.[19] The use of medium-acting glucocorticoids at doses 2-3 times divided can induce and sustain hyperglycemia throughout the day.[20] To address the patient's hyperglycemia, a rapid insulin infusion of 3 IU/hour is administered.

The effects of glucocorticoids on glucose metabolism likely stem from the disruption of multiple pathways, including beta cell dysfunction (sensitivity to glucose and insulin release) and insulin resistance in various tissues. The impact on beta cell function and insulin sensitivity may differ depending on whether glucocorticoid effects are acute or chronic. A study comparing a single acute dose of prednisolone (75 mg) with a daily dose of 30 mg for 15 days revealed that acute treatment inhibits several parameters of beta cell function. In contrast, prolonged exposure to glucocorticoids demonstrated partial recovery of beta cell function, yet glucose tolerance was impaired, suggesting additional factors play a role in steroid-induced diabetes mellitus (SIDM) beyond beta cell dysfunction.[21]

Several mechanisms underlie the diabetogenic effects of glucocorticoids, contributing to hyperglycemia. These include the reduction of peripheral insulin sensitivity, elevation of hepatic gluconeogenesis, induction of insulin resistance in lipid metabolism and adipose tissue, and inhibition of pancreatic insulin production

and secretion. Glucocorticoids pose the highest risk for hyperglycemia and the development of overt diabetes mellitus.[22]

In addition to the duration of exposure, the potency-related duration of action of glucocorticoids is a crucial factor influencing the severity of post-glucocorticoid hyperglycemia. Research by Yasuda et al. in 1982 demonstrated that hydrocortisone, dexamethasone, and prednisone induced varying degrees of insulin resistance, primarily through a decrease in insulin binding affinity rather than a reduction in the number of receptors.[23]

Glucocorticoids directly impact glycogen synthesis pathways, insulin-mediated degradation, and protein synthesis. The primary site for insulin-mediated glucose uptake is skeletal muscle. Insulin facilitates the recruitment of the glucose transporter (GLUT-4) to the cell surface, enabling the uptake of glucose into the cell. Glucocorticoids disrupt insulin-mediated glucose uptake by directly interfering with key components of the insulin signaling cascade, including glycogen synthase kinase-3, glycogen synthase, and GLUT-4 translocation.[24]

The management of corticosteroid-induced hyperglycemia differs from that of non-steroid-related diabetes. Insulin sensitizers such as metformin, commonly used as a first-line treatment for type 2 diabetes mellitus, are not recommended for steroid-induced diabetes mellitus (SIDM). This is attributed to relative or absolute contraindications to metformin use, including nausea/vomiting, hypoxia, liver disorders, and kidney disorders.[12]

In outpatient management, certain oral hypoglycemic drugs (OHO) show the potential to improve glycemic control and prevent or delay the onset of corticosteroid-induced hyperglycemia.[25] However, there is limited evidence demonstrating the clinical effectiveness of OHO use for in-hospital hyperglycemia caused by glucocorticoids.[26]

Management strategies for controlling hyperglycemia induced by glucocorticoids have been investigated in various studies. The hyperglycemic effects of different glucocorticoids influence the pharmacokinetic profile of the glucocorticoid. Therefore, the choice of insulin therapy in SIDM must take into account the specific glucocorticoid therapy, its current dose, and the timing and intervals of administration.[12] NPH insulin, based on body weight, was selected due to its duration of action being similar to that of medium-acting glucocorticoids, thereby mitigating the risk of hypoglycemia once the steroid effect diminishes.

For corticosteroids administered twice daily, long-acting insulin such as glargine or detemir may be preferable during periods of hyperglycemia.[19] The 2012 Endocrine Society guideline recommends initiating insulin at 0.3-0.5 units/kg/day when using glucocorticoids, with no specific recommendations regarding the proportion of basal and bolus insulin.[27] Suh's research in 2017 suggested an NPH insulin dose of 10% of the corticosteroid prednisone dose.[18] Thus, adjustments to basal and bolus insulin doses are necessary to manage hyperglycemia. The target for monitoring pre-prandial blood sugar is < 140 mg/dL, and < 180 mg/dL for post-prandial sugar.

When hyperglycemia due to steroids is not controlled with oral hypoglycemic drugs, insulin may be administered once a day or through a more complex insulin regimen 2-3 times a day, such as premixed insulin or basal-bolus insulin. Insulin dose titration is crucial for maintaining glycemic control by adjusting the insulin dose as needed. In critically ill patients with severe hyperglycemia, intravenous insulin infusion (drip insulin) may be required.[28]

The initiation of glucocorticoids may induce postprandial hyperglycemia, while the reduction of glucocorticoids may result in the normalization of glycemic control. Combination basal-bolus insulin therapy, incorporating basal insulin, prandial insulin, and an additional correction factor insulin, remains the most flexible option for patients.[29] However, conventional use of high doses of long-acting basal insulin can lead to nocturnal hypoglycemia.[30]

CONCLUSION

In conclusion, the hyperglycemia induced by glucocorticoids was identified through postprandial sugar monitoring, necessitating treatment through modifications to basal and bolus insulin doses. Strategies for managing hyperglycemia should be tailored to the pharmacokinetics of glucocorticoids and insulin.

AUTHORS' CONTRIBUTIONS

Fatimatuz Zahro and Tita Sugesti took research data and wrote this journal. Siska Hermawati, M. Hari Pristantiningtyas, Herya Putra Dharma and Muhammad Muchlis chose cases in the hospital that could be used as case reports, as well as guiding the writing of this journal. Jainuri Erik Pratama, Fauna Herawati, Adji Prayitno Setiadi and Marisca Evalina Gondokesumo reviewed and supervised the journal. All authors have read and approved the final journal.

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JTL 13 (1): 2024
Page: 44-52

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Original Research

Bidara Leaf Extract (Ziziphus mauritiana L.): A Natural Approach to Enhancing Pancreatic Function and Lowering Blood Sugar in Male Wistar Rats

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Abstract: Diabetes mellitus is a metabolic disorder characterized by insufficient insulin production, leading to elevated blood glucose levels. When pancreatic beta cells produce inadequate insulin to compensate for increased insulin resistance, chronic hyperglycaemia ensues. This study investigates the effect of Bidara leaf extract (*Ziziphus mauritiana*) on the pancreatic function of male Wistar rats and its efficacy in reducing blood sugar levels. The experimental design was a post-test only controlled group study. Six groups were included: a control group receiving 1% Na CMC and five treatment groups receiving Bidara leaf ethanol extract at doses of 50 mg/kg, 100 mg/kg, 300 mg/kg, and 500 mg/kg, with each group consisting of four rats. Data analysis involved normality tests, homogeneity tests, and ANOVA. Results indicated that the p-value for each group was less than 0.05. Specifically, the 50 mg/kg dose of Bidara leaf extract significantly reduced blood sugar levels ($p = 0.02$). The 100 mg/kg dose showed a p-value of 0.14, indicating effectiveness in lowering blood sugar levels, though less pronounced. Similarly, the 300 mg/kg dose ($p = 0.16$) and the 500 mg/kg dose ($p = 0.04$) demonstrated significant hypoglycaemic effects. In conclusion, Bidara leaf extract has a dose-dependent effect on lowering blood sugar levels in male Wistar rats, with significant results observed at varying concentrations. Further research is recommended to explore the mechanisms involved and potential therapeutic applications in humans.

Keywords: *Diabetes Mellitus; Bidara Leaf Extract; Ziziphus mauritiana; Blood Sugar Levels; Pancreatic Function.*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that is unable to produce enough insulin, resulting in high glucose levels in the blood[1,2]. High levels of glucose in the blood are caused by the body not being able to convert glucose or carbohydrates into energy because the body no longer produces enough insulin or the body no longer produces insulin which causes glucose to not be able to enter the cells to be converted into energy and there is an increase in blood glucose levels, resulting in damage to various body tissues ranging from blood vessels, eyes, kidneys, heart, and also nerves[3,4].

Diabetes mellitus is a worldwide health problem and the number of sufferers continues to increase. The global status report issued by the World Health Organization (WHO) states that the prevalence of diabetes mellitus worldwide is estimated at 9%. Meanwhile, the proportion of deaths caused by diabetes mellitus from all deaths caused by non-communicable diseases is 4% [5]. Deaths caused by diabetes mellitus occur in low and middle income countries with a proportion of 80%. By 2030, it is estimated that diabetes mellitus will be the 7th leading cause of death in the world[6].

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Indonesia is the 7th country with the highest prevalence of diabetes, after China, India, the United States, Brazil, Russia and Mexico[7]. Asia states that out of 1,785 people in Indonesia who experience DM. The 2018 Riskesdas (Primary Health Research in Indonesia) report shows that the prevalence of DM diagnosed by doctors in the population aged ≥ 15 years is 2%. In 2021 Indonesia rose to 5th place with the highest number of DM cases with a prevalence of 19.5 [8].

One of the plants used as a medicine by the community is herbal bidara plant (*Ziziphus mauritiana Lam*.) is one of the plants found in Indonesia and its properties are not widely known[9,10]. Bidara leaves contain several compounds such as alkaloids, flavonoids, quercetin, phenols, rutin, and terpenoids that play a significant role in increasing antioxidant activity and are able to regenerate damaged pancreatic β cells[11]. Flavonoids are also thought to improve the sensitivity of insulin receptors, which is beneficial in DM[12].

Research conducted by Haeria, 2016 concluded that bidara leaf extract has strong antioxidant activity, this is due to the flavonoid content contained in it. Flavonoids are reducing compounds that can inhibit many oxidation reactions by transferring electrons to free radical compounds so that the free radical compounds become stable and without oxidation reactions occurring[13].

MATERIAL AND METHOD

This type of research is experimental using a pre-test and post-test control group design[14]. The research was conducted at the Pharmacology Laboratory of the Faculty of Pharmacy, University of North Sumatra from September 2023 to December 2023. The experimental animal research protocol was approved by the Prima Indonesia University Health Research Ethics Committee (012/KEPK/UNPRI/IX/2023). The test animals used in this study were healthy Wistar male rats obtained from Ellio Sains Laboratory. The 24 rats used were divided into 6 groups using bidara leaf ethanol extract 50, 150, 300 and 500 mg/KgBB and 1% Na-CMC as negative control and positive control was Metformin 500mg/KgBB.

Tools and Materials

The tools used in this research are measuring cup (Pyrex), drop pipette, sudip, analytical balance (*Mettler Toledo*), maceration vessel, stirring rod, rotary evaporator, jar. The materials used in this study are bidara leaves (*Ziziphus Mauritiana L*.), 96% ethanol, 1% Na CMC, Alloxan, Metformin, wistar male rats, husks for rat cages, rat feed, rat drinking water, and rat food.

Bidara leaf extraction (*Ziziphus mauritiana L*.)

Making bidara leaf extraction using maceration method. Maceration uses 96% ethanol solvent. Bidara leaf simplisia powder weighed 500 grams was soaked using 96% ethanol solvent as much as 3.75 liters for 3x24 hours while stirring occasionally at the same time, then the filtering process was carried out. The macerate was then filtered first using filter paper and evaporated using a waterbath at 60oC until a thick extract was obtained[15,16].

Blood Collection Process

Blood sampling is done by surgery to obtain blood from the heart of Wistar rats. Blood collection from the heart aims to get more blood volume compared to blood collection in the tail and eyes of rats. Before being dissected, the rats were first fed for 12 hours. Then the rats were euthanized until the rats lost consciousness (fainting). The rats were placed on a surgical tray (supine position) using a needle to puncture the legs so that the rats were spread-eagled. Make a midline incision in the abdominal wall muscle from the end of the stenum to the pubic symphysis. In the midline cut there will be a little bleeding. The cut in the abdominal muscle does not hit the diaphragm to avoid pneumothorax. Locate the

heart on the left side of the chest between the 3rd and 4th costae next to the sinister sternum. Insert the syringe into the heart 5 mm deep from the thorax towards the chin. The syringe forms an angle of 25-30° from the rat's chest. Blood was immediately drawn as much as 5 ml using a syringe and collected in a vacutainer[17].

Treatment Procedure

Animals in 1 group were placed together in 1 cage. Groups 3 to 6 were given ethanol extract of Bidara leaves (*Ziziphus Mauritiana L.*) orally according to the dose level while the negative control group was given 1% Na CMC solution and the positive control was given metformin 500 mg/KgBB. Grouping was divided into: Group I was given 1% Na CMC as a negative control, group II was given Metformin 500mg/KgBB as a positive control (*Ziziphus mauritiana* L), group III was given 50 mg/KgBB of bidara leaf ethanol extract (*Ziziphus mauritiana* L), group IV was given 150 mg/KgBB of bidara leaf ethanol extract (*Ziziphus mauritiana* L), group V was given 300 mg/KgBB of bidara leaf ethanol extract (*Ziziphus mauritiana* L) and group VI: 500 mg/KgBB of ethanol extract of bidara leaves (*Ziziphus mauritiana* L). Initial blood sugar checks were carried out on day 0, then after checking the blood glucose levels of normal rats, the rats were fed for 8 hours. Rats were induced alloxan intra peritoneally with a total dose of 24 mg on day 1, and diabetic blood sugar was checked on day 3. Rats were declared hyperglycemia if the blood glucose level was ≥ 135 mg/dL [18,19].

Histopathologic Examination

Pancreas samples were taken on the 28th day of the test animals. The pancreas obtained was then cleaned and fixed with 10% (Buffer Formaline Bio Analitika Pro Analysis) solution for at least 24 hours, then the pancreas samples were dehydrated with graded alcohol concentrations, followed by clearing using xylol, impregnation and embedding using paraffin. Blocks were cut to 5µm thick with a microtome, then Hematoxylin-Eosin (HE) general staining was performed[20,21].

Data Analysis

The research data were analyzed using statistical program. Data normality test was analyzed by Shapiro Wilk test ($p > 0.05$). Further analysis were carried out using paired Sample T - Test to see the relationship between each dose to reduce blood sugar levels[22,23].

RESULTS AND DISCUSSION

Phytochemical screening test results of bidara leaf (*Ziziphus mauritiana* L.)

Phytochemical screening is a qualitative test used as an initial stage carried out on bidara leaf extract (Ziziphus mauritiana L.). The purpose of phytochemical screening is to determine the secondary metabolite compounds contained in bidara leaf extract. The results of phytochemical screening can be seen in Table 1.

Table 1. Compounds contained in Bidara Leaf (*Ziziphus mauritiana* L.)

Secondary Metabolite Compounds	Reagents	Result
Alkaloid	Bouchardart	-
	Maeyer	-
	Dragendroff	+
	Wagner	+
Terpenoids/steroids	Salkowsky	-
	Lieberman-Burchad	-
Saponins	Aquadest + 96% Alcohol	+
Flavonoids	$Mg_{(s)}+HCl_{(p)}$	+
Tannin	$FeCL_3$ 1%	+
Glycosides	Mollish	+

Effectiveness of Bidara Leaf Extract at a Level of 50, 150, 300 and 500 mg/KgBW in Reducing Blood Sugar Levels

The statistical analysis results for the effectiveness of Bidara leaf extract at concentrations of 50, 150, 300, and 500 mg/kgBW in reducing blood sugar levels reveal significant p-values of 0.002, 0.014, 0.016, and 0.004, respectively. This indicates a statistically significant reduction in blood sugar levels at all tested concentrations. The lowest concentration of 50 mg/kgBW demonstrated a highly significant effect with a p-value of 0.002, suggesting that even minimal doses of the extract can effectively lower blood sugar levels. At 150 mg/kgBW, the extract continued to show significant hypoglycemic effects with a p-value of 0.014, likely due to the presence of bioactive compounds such as flavonoids that enhance insulin sensitivity and provide antioxidant benefits. The concentration of 300 mg/kgBW also maintained its effectiveness in reducing blood glucose levels, with a p-value of 0.016, indicating a consistent beneficial impact across varying dosages. The most substantial effect was observed at the highest concentration of 500 mg/kgBW, with a p-value of 0.004, underscoring the extract's potent hypoglycemic properties at higher doses. This dose-dependent response highlights the potential of Bidara leaf extract as a therapeutic agent for diabetes mellitus, demonstrating significant efficacy in lowering blood sugar levels across all tested concentrations.

Histopathology of pancreatic β-cells in Wistar Rat

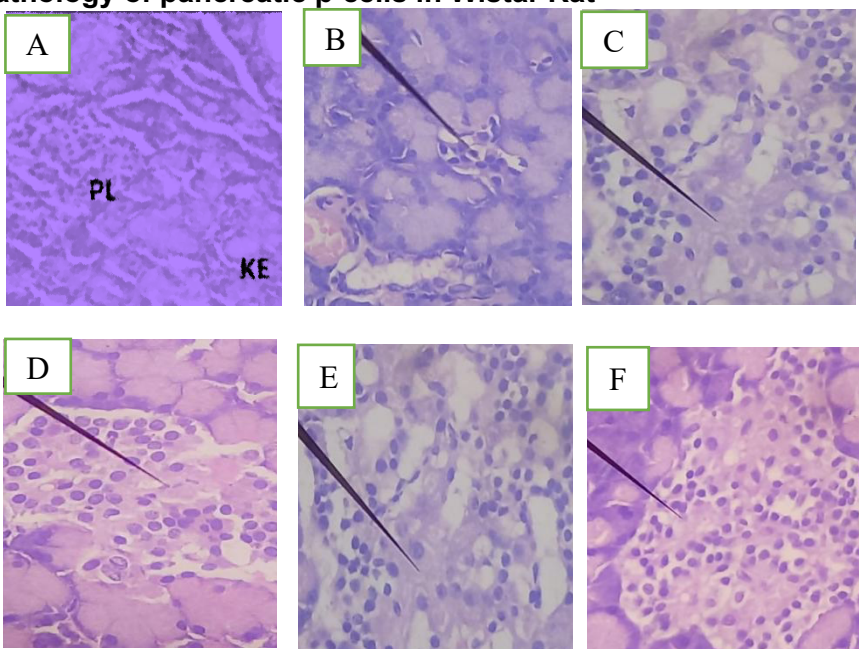

Figure 1. Positive Control (A); Negative Control (B); Dose 50 mg/KgBB (C); Dose 150 mg/KgBB (D); Dose 300 mg/KgBB (E); Dose 500 mg/KgBB (F)

The study investigates the effects of various doses of Bidara leaf extract on the Langerhans islets and histopathological changes in the pancreas of male Wistar rats, with comparisons to positive and negative controls. For the Positive Control group (A), diffuse expansion between the interlobular septum was observed, accompanied by inflammation around the ductal periphery. However, there was no vascularization, and necrosis was limited to 5-10 necrotizing cells per low-power field (LPF), indicating a relatively mild degree of pancreatic damage.

In the Negative Control group (B), the Langerhans islets value at 10x10 magnification was 7. Histopathological analysis at 10x40 magnification revealed

significant damage, with 50% necrosis and 70% degeneration of the pancreatic tissue, indicating substantial pancreatic impairment without any treatment.

At a dose of 50 mg/kgBW (C), the Langerhans islets value decreased to 5 at 10x10 magnification. Histopathological examination showed 80% necrosis and 70% degeneration at 10x40 magnification, suggesting that this low dose was not sufficient to protect the pancreas and may have contributed to increased tissue damage. For the 150 mg/kgBW dose group (D), the Langerhans islets value further decreased to 3 at 10x10 magnification. Necrosis remained at 80%, and degeneration increased to 80% at 10x40 magnification, indicating persistent and severe pancreatic damage at this intermediate dose. In the 300 mg/kgBW dose group (E), the Langerhans islets value improved to 7 at 10x10 magnification, matching the negative control group. Histopathological analysis revealed 50% necrosis and 70% degeneration at 10x40 magnification, showing that this dose reduced necrosis but did not significantly affect degeneration compared to the negative control.

At the highest dose of 500 mg/kgBW (F), the Langerhans islets value significantly increased to 12 at 10x10 magnification. Histopathological results showed 50% necrosis and 70% degeneration at 10x40 magnification, indicating that this dose provided the most substantial protection against pancreatic damage, evidenced by a higher number of Langerhans islets and reduced necrosis compared to lower doses and the negative control.

In summary, the results suggest that Bidara leaf extract exhibits a dose-dependent effect on pancreatic protection. Lower doses (50 and 150 mg/kgBW) were associated with higher necrosis and degeneration, while higher doses (300 and 500 mg/kgBW) showed improved pancreatic health, with the 500 mg/kgBW dose being the most effective in preserving Langerhans islets and reducing necrosis. These findings indicate that higher doses of Bidara leaf extract may have therapeutic potential in mitigating pancreatic damage and managing diabetes mellitus. Further research is needed to elucidate the mechanisms underlying these protective effects and to confirm their applicability in clinical settings.

The results of the study after being given standard feed plus induced Alloxan intraperitonially or intravenously once for 7 days and increased body weight and blood glucose. The mean blood glucose level in white rat before hyperglycemia was 165.2 mg/dL, while the mean blood glucose level in white rat after hyperglycemia was 308 mg/dL. This shows that there is an increase in blood glucose levels in white wistar rats by 163.8 mg/dL. The increase in blood glucose levels on alloxan administration can be caused by two processes, namely the formation of free radicals and damage to cell membrane permeability resulting in damage to pancreatic beta cells that function to produce insulin[24]. The toxic action of alloxan on beta cells is initiated by free radicals formed by redox reactions. Alloxan and its reduction product, dialuric acid, form a redox cycle with the formation of superoxide radicals[25]. This radical undergoes dismutation to hydrogen peroxide. Hydroxyl radicals with high reactivity are formed by the Fenton reaction [26]. Free radical action with high excitability increases cytosolic calcium concentration leading to rapid destruction of pancreatic beta cells. The increased cytosolic calcium concentration is also due to alloxan inducing calcium release from the mitochondria which then leads to disruption of the oxidation process of pancreatic beta cells[27].

Examination of blood glucose levels was carried out before and after the administration of bidara The examination of blood glucose levels was carried out before and after the administration of bidara leaf ethanol extract using six treatment groups of bidara leaf ethanol extract 50, 150, 300 and 500 mg/KgBB and Na-CMC 1% as negative control and positive control is Metformin 500mg/KgBB which has the effect of lowering blood sugar levels in rats. The decrease in blood glucose levels can be caused by the anthocyanins contained in bidara leaves. Anthocyanins include flavonoid group pigments that produce orange, red and blue

colors that are soluble in water and easily degraded. Anthocyanin degradation can be caused by pH, light, temperature, and the addition of sugar. Anthocyanins are high enough as antioxidants that can reduce the risk of diabetes mellitus. It is known that dietary antioxidants, including anthocyanins, protect β-pancreatic cells from glucose induced oxidative stress[28,29].

According to Maulana's research, 2020 bidara leaf extract contains antidiabetic activity. Bidara leaf extract is obtained through the mechanism of inhibiting enzymes that break carbohydrates into glucose found in the gastrointestinal tract, two groups of enzymes that are inhibited are α-Amylase and α-Glucosidase. The α-amylase enzyme group is produced by the salivary glands and pancreas whose main function is to break down amylum (salivary amylase) and break down glycogen (pancreatic amylase), inhibition of its activity will inhibit the breakdown of carbohydrates in the gastrointestinal tract and in the body, thus affecting the availability of glucose in the blood plasma. The α-Glucosidase group, including maltase, isomaltase, glucomaltase, and sucrase, has the function of hydrolyzing oligosaccharides that enter the small intestine, so if inhibited, it will affect the digestion of carbohydrates and their absorption so as to prevent an increase in blood glucose levels after eating[30].

CONCLUSION

The study on Bidara leaf extract's effects revealed significant findings regarding its potential as a therapeutic agent for diabetes mellitus. The extract demonstrated dose-dependent hypoglycemic effects across concentrations of 50, 150, 300, and 500 mg/kgBW, with all doses showing statistically significant reductions in blood glucose levels. Particularly notable was the substantial efficacy at the lowest dose of 50 mg/kgBW, indicating potent hypoglycemic properties. Histopathological analysis showed that higher doses (300 and 500 mg/kgBW) preserved Langerhans islets and reduced pancreatic necrosis compared to lower doses and controls, suggesting protective effects against diabetes-induced pancreatic damage. These results highlight Bidara leaf extract's potential in enhancing insulin sensitivity, scavenging free radicals, and protecting pancreatic beta cells from oxidative stress. Further research is needed to fully understand its mechanisms and confirm its clinical applicability in managing diabetes. Bidara leaf extract stands out as a natural remedy with promising therapeutic benefits, warranting continued investigation into its pharmacological properties and safety profile.

AUTHORS' CONTRIBUTIONS

Musdayani Nasution prepared the samples, designed the protocols, executed the protocols, and wrote the manuscript. Ali Napiah Nasution and Maya Sari Mutia reviewed and supervised the manuscript. All authors have read and approved the final manuscript

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigastion are available from the corresponding author on reasonable request

DISCLOSURE STATEMENT

There is no conflict of interest.

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JTL 13 (1) MAY 2024
Page: 33-43

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Original Research

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Antioxidant protective effect of Nelumbo nucifera extract against spermatogenic cells in male mice due to 2-methoxyethanol exposure

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Abstract: One of the causes of infertility in men is influenced by the compound *2-Methoxyethanol* (2-ME) that can increase *Reactive Oxygen Species* (ROS) which can distrub spermatogenesis. This study aimed to analyze the effect of antioxidant of *Nelumbo nucifera* extract on the histology of mice testicular spermatogenic cells. Male mice are divided into 6 groups. The negative control group (KN) was given distillated for 28 days *ad libitum*. The positive control group (KP) was injected subcutaneous (s.c) with 2-ME dose 200 mg/kg bw as much as 0.05 ml/ day for 7 days, the drug group (KO) was injected s.c with 2-ME (200 mg/kg bw) as much as 0.05 ml/ day for 7 days and then injected s.c with *Clomiphene citrate* with a dose 50 mg/kg bw as much as 0.2 ml/ day for 21 days. The treatment group (P1, P2, P3) was injected s.c with 2-ME (200 mg/kg bw) as much as 0.05 ml/ day for 7 days and injected s.c with *N. nucifera* extract at low dose 50 mg/kg bw (P1), medium dose 150 mg/kg bw (P2), and high dose 450 mg/kg bw (P3) as much as 0.2 ml/ day for 21 days. At the end of the study all mice were sacrificed and testicular collection was carried out. Testicular tissue was processed using Hematoxylin-Eosin staining and observed spermatogenic cells (spermatogonia, spermatocytes and spermatids). Data analysis using ANOVA test and advanced test Post hoct test. The results showed that there was a significant difference ($p < 0.05$) in number of spermatogenic cells of mice testicles between the negative group (KN), positive group (KP) and treatment group (P1, P2, P3). The optimal dose of *N. nucifera* extract that is most able to repair testicular tissue damage is a high dose.

Keywords: Antioxidant; Spermatogenic cells; histopatology, *Nelumbo nucifera*; 2-*Metoxyethanol*

INTRODUCTION

The prevalence of infertility in Indonesia is increasing every year, based on data there were 1,712 men and 2,055 women who experienced infertility[1]. Infertility is not only experienced by women but, in this case the male factor is responsible for 36% while 64% is in women[2]. Infertility in men is influenced by several factors, one of which is declining sperm quality, both in terms of sperm count, sperm morphology, and sperm motility[3]. 2-*methoxyethanol* (2-ME) is one example of a glycol ether compound also known as *ethylene glycol monomethyl ether* (EGME). This compound is often used as an organic solvent to produce various industrial materials, such as resins, wood paints, wall paints, varnishes, nail polishes, and

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cellulose acetate, however, 2-ME is highly toxic to tissues that proliferate rapidly and have a high metabolic rate, such as the testes and thymus [4,5].

The process of spermatogenesis can be disrupted if exposed to excessive exposure to free radicals by damaging cell membranes. This compound can cause damage to *Deoxyribonucleic Acid* (DNA) spermatozoa and apoptosis of spermatozoa cells[6]. Sperm quality can affect a man's fertility potential. Oxidative stress is capable of decreasing the physiological capacity of fertilization, including capacitation, acrosome reactions, hyperactivation and sperm-oocyte binding, which will result in male infertility. Male fertility potential can be improved through the prevention and management of oxidative stress. Consumption of antioxidants in large quantities is a major factor to prevent oxidative stress[7]. Antioxidants are compounds that can prevent oxidative stress, one of the natural antioxidants is flavonoids. Flavonoids, which act as antioxidants, have the ability to inhibit ROS formation by inhibiting redox reactions that produce fresh oxidants[8].

Ethanol extract from *Nelumbo nucifera* revealed that this plant contains important phytochemical components phenols, flavonoids, tannins, alkaloids, saponins, steroids, terpenoids, cardiac glycosides, coumarins and quinones[9]. Flavonoids are one of the powerful antioxidants that function as free radical chain breaking antioxidants[10]. Flavonoids will capture free radicals by releasing hydrogen atoms from their hydroxyl groups and breaking the free radical chain reaction[11]. The flavonoids compound can affect the reproductive process including spermatogenesis because it acts as free radical scavenging that causes infertility. Antifertility drugs have a mechanism of action through cytotoxic or cytostatic effects[12]. Spermatogenesis in mice takes 35.5 days to complete in one cycle, or 4 times longer than the seminiferous epithelial cycle. Proliferation, growth, maturation, and transformation or spermiogenesis are different phases of spermatogenesis[13]. Spermatogenesis is the process of producing sperm from spermatogonium, through complex and orderly development. Spermatogenesis occurs within the seminiferous tubules of the testes, through a series of processes such as proliferation, differentiation and transformation[14].

This study aimed to explore the potential of *Nelumbo nucifera* extract in protecting and repairing sperm damage due to 2-ME exposure. This study was intended to identify optimal doses of *Nelumbo nucifera* extracts with potential compounds and identify potential compounds as anti-infertility drug candidates. In this context, this research has significant relevance in the field of phytopharmaceuticals and may contribute to the understanding of the potential use of *Nelumbo nucifera* as a natural alternative medicine to address infertility problems in men.

MATERIAL AND METHOD

Ingredients used include *Nelumbo nucifera* extract, male mice strain ddy, filter paper, ethanol 100%, ethanol 96%, ethanol 95%, ethanol 90%, ethanol 80%, ethanol 70% and ethanol 50%, distilled water, 2-*Methoxyethanol*, *Neutral Formalin Buffer* (NBF 10%), parafin (liquid), 2,2-difenil-1-pikrilhidrazi (DPPH), chloroform, normal buffer formaline (NBF) 10%, xylol, hematoxylin-eosin tissue dye. tools used include analytical balances, beers, stirrers, pipettes, petri dishes, 1cc syringes, Eppendorf tubes, object glass, glass covers, slide boxes, digital scales, probes, light microscopes, micropipettes, mice cages with food and water, Pasteur pipettes, Eppendorf tubes, Neubauer counting chambers, rotary vacuum evaporators, and water baths.

Ethical Approval

All procedures in this study, including the use of mice as animal models, have been approved by the Ethics Committee, Department of research and Community Services, Universitas Brawijaya, East Java, Indonesia, with number No: 370-KEP-UB-2023.

Plant material
Nelumbo nucifera were collected from Jotosanur Reservoir, Lamongan, East Java. The material was identified and authenticated in the Biology Laboratory, Department of Biology, Universitas Muhammadiyah Lamongan.

Preparation of ethanol extract and dosage of *Nelumbo Nucifera*
Nelumbo nucifera are cut into small pieces and dried in an oven at 40°C for 60 Minutes. Dried (500 grams) are electrically ground and macerated with 96% ethanol for 3 days at room temperature. The extract is then filtered and concentrated with a rotary evaporator and heated in a water bath at a temperature of 70°C. The dosage used are 50, 150, and 450 mg/kg bw. Each mice receive 0,02 ml/day for twenty one days[15].

Preparation of 2-*Methoxyethanol* suspension
For a seven-day stock of 200 mg/kg bw per individual mice, thirty five milligrams of 2-*Methoxyethanol* are weighed and dissolved in 0,35 mL of Na-CMC solution. Each mice receive 0,05 ml/day[15].

Prepartion of *Clomiphene citrate* drug suspension
For a twenty one day stock of 50 mg/kg bw per individual mice, ten milligrams of chlomiphene citrate are weighed and dissolved in 4,2 mL of Na-CMC solution. Each mice receive 0,2 ml/day[15].

Animals
Thirty six adult male mice of ddy strain aged 6-7 weeks, weighing 25-30 grams, were obtained from the Center for Animal Health and pharmacy, Pusvetma, Surabaya, East Java. They were kept in standard laboratory conditions (temperature 28-30°C, 12-hour/12-hour light/dark cycle) and fed and watered *ad libitum*.

Experimental design
After one week of acclimatization, the mice were randomly divided into six equally large groups (n=6) as follows: the negative control group (KN) received distilled water for 28 days *ad libitum*, the positive control group (KP) received subcutaneous injections of 0.05 ml of 2-ME at a dose of 200 mg/kg bw for 7 days. The drug group (KO) received subcutaneous injections of 0.05 ml of 2-ME for 7 days and received subcutaneous injection of 0,2 ml of *Clomiphene citrate* at a dose 50 mg/kg bw for 21 days. The treatment group (P1, P2, and P3) received subcutaneous injections of 0.05 ml of 2-ME at a dose of 200 mg/kg bw for 7 days. Subsequently, subcutaneous injections of 0.2 ml at different doses of *N. nucifera*. The first treatment (P1) received a low dose (50 mg/kg bw), the second group (P2) received a medium dose (150 mg/kg bw), and the third group (P3) received a high dose (450 mg/kg bw) for 21 days. After the procedure, all mice were sacrificed using chloroform. The testis organ were weighed, and fixed into NBF 10% for histopatological analysis.

Antioxidant test of *Nelumbo nucifera* extract
Conducted in vitro using the method of suppression of free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH). The test was conducted using UV-Vis spectrophotometry measured its absorption at a wavelength of 517 nm[16].

Antioxidant data analysis
The percentage of inhibition is the presentation that indicates the activity of the radical. Presentation of inhibition of DPPH-free radical from each concentration of solution the sample can be calculated by the formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of treatment}}{\text{Absorbance of control}} \text{ X } 100$$

Description: absorbance of control = absorbance on DPPH without sample, absorbance of treatment = absorbance on DPPH after adding the sample. The IC50 value of 50% ethanol extract was determined by determining the relationship between the concentration and the percentage of inhibition with the equation y= ax +b. the X axis is the sample concentration (ppm) and the y is the percentage of inhibition[17].

Histology preparation

Fixed testes in a 10% *neutral buffer formaline* (NBF) solution, then made histological preparations using paraffin method and hematoxylin eosin staining with a thickness of 3-5 µm[18].

Histological research parameters

The histological preparation of the testicular organ is examined under a microscope with a magnification of 400x. The seminiferous tubules look round in shape. The number of spermatogenic cells (spermatogonia, spermatocytes and spermatids) is a measured parameter. Cell count data collection was conducted by observing 5 field of view of fully spherical seminiferous tubules for each treatment and test. Then, a photo taking of the histological preparation takes place[19].

Analysis of histological data

The test results of the effect of *Nelumbo nucifera* extract on the number of spermatogenic cells in mice were analyzed using *One Way analysis of Variance* (ANOVA) and Post Hoct test with SPSS software, 95% confidence level (α =0.05%).

RESULTS AND DISCUSSION

Antioxidant Activity Test

Measurement of DPPH test results and obtained the value of percentage of inhibition of each concentration (Inhibition concentration/ IC50). IC50 value of ethanol extract of *Nelumbo nucifera* obtained 105.8 ppm. These values can be seen in (Table 1).

Table 1. Antioxidant Test Results of *Nelumbo nucifera* Extract

Concentration (ppm)	Absorbance (Abs)	percentage of inhibition (%)	IC_{50} (ppm)
20	0,156	0,87	
40	0,305	0,75	
60	0,376	0,69	105,8
80	0,447	0,64	
100	0,560	0,54	
Concentration (ppm)	Absorbance	percentage of inhibition (%)	IC_{50}

The calculation results with simple linear regression analysis can be presented in (Figure 1) with the IC50 value obtained from the equation y = 0.475x + 0.0838. The x value is the IC50 value and the y value is 50. The classification of antioxidants is divided into 5, namely <50 ppm (very strong), 50-100 ppm (strong), 100-150 ppm (medium), 150-200 ppm (weak) and >200 ppm is very weak[20]. The linear regression equation also shows that there is a significant relationship

between the solvent concentration and the percentage of inhibition indicated by the degree of tightness x. Based on the results of the study, the IC50 value was obtained from *Nelumbo nucifera* ethanol extract, which is 105.8 ppm, *Nelumbo Nucifera* extract has moderate antioxidant activity.

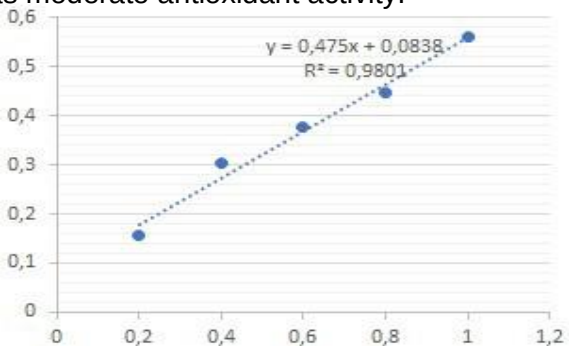

Figure 1. Regression values on the antioxidant activity graph

Mice Testicle Weight

The results of weighing the testes of mice that have been given *Nelumbo nucifera* extract after induced 2 ME there is a difference in weight in each group (Table 2). In the positive group (KP) has an average of the smallest testicular weight and in the negative group (KN) has an average value of the largest testicular weight as well as the number of spermatogenic cells.

Table 2. Testicle weight of mice that have been given *Nelumbo nucifera* extract after induction of 2-*Methoxyethanol*

Treatment	Total	Average of testicular weight (gram)
KN	5	0,358
KP	5	0,195
KO	5	0,310
P1	5	0,220
P2	5	0,260
P3	5	0,320

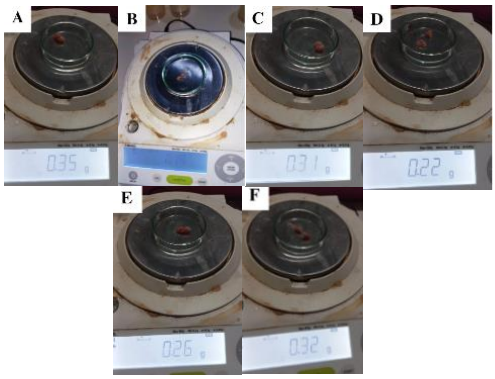

Figure 2. Weight of mice testes after induction of 2-*Methoxyethanol* and *Nelumbo nucifera* extract administration. Description: A: negative control (KN); B: Positive Group (KP); C: Drug Control: D: first treatment (P1: low dose *Nelumbo nucifera* of extract): E: second treatment (P2: medium dose *Nelumbo nucifera* of extract); F: third first treatment (P3: high dose *Nelumbo nucifera* of extract).

Histological Tests of The Testes of Mice

The results showed that the number of spermatogonia cells, spermatocytes, and spermatid cells in the positive control induced by 2-Methoxyethanol had lower values than the negative control (normal) and the group treated with *Nelumbo Nucifera* extract (Table 3). These results prove that 2-ME induction causes cell damage.

Table 3. Effect of *Nelumbo nucifera* extract after induction of 2-*Methoxyethanol* on the number of spermatogonia cells, spermatocytes, and spermatids

Treatment	Spermatogonia Cells	spermatocyte Cells	Spermatid Cells
KN	117,8 ± 3,99 [a]	120,8 ± 5,09 [a]	145,6 ± 4,77 [a]
KP	29,2 ± 3,99 [b]	25,4 ± 5,09 [b]	2,2 ± 4,77 [b]
KO	76,4 ± 3,99 [c]	116,4 ± 5,09 [a]	137,4 ± 4,77 [a]
P1	56,6 ± 3,99 [d]	62 ± 5,09 [c]	85 ± 4,77 [c]
P2	73 ± 3,99 [c]	85 ± 5,09 [d]	105,8 ± 4,77 [a]
P3	118,6 ± 3,99 [a]	115 ± 5,09 [a]	135,6 ± 4,77 [a]

Description: superscript differences in the same column indicate significant differences ($p<0.05$). The Data are presented in the mean of the ± SD

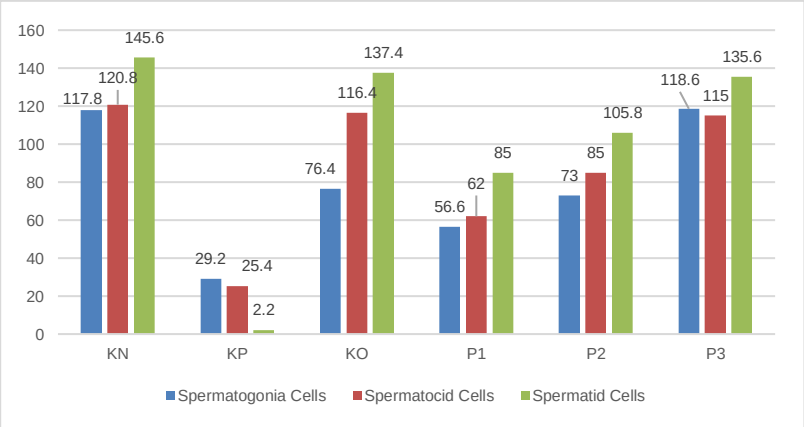

Figure 3. Histogram of spermatogenic cells number among control and treatment group. KN: negative control, KP: Positive Group, K0: Drug Control, (P1): low dose of *Nelumbo nucifera* extract (P2): medium dose of *Nelumbo nucifera* extract, (P3): high dose of *Nelumbo nucifera* extract.

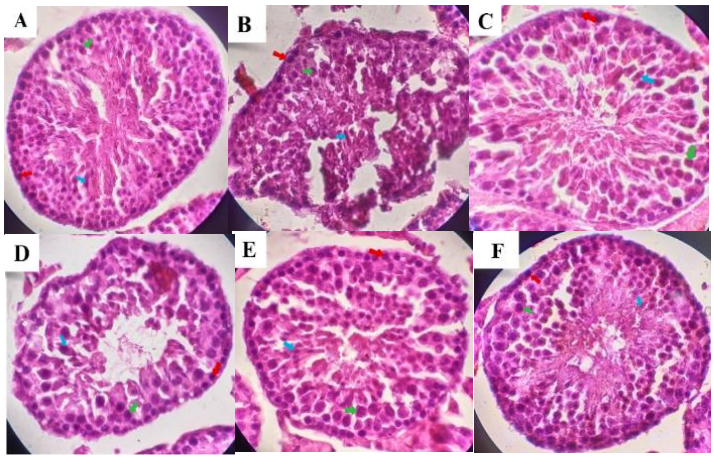

Figure 4. Cross-section of spermatogenic mice after treatment with extract *Nelumbo nucifera*. Description: A: negative control (KN); B: Positive Group (KP); C: Drug Control (KO): D: first treatment (P1: low dose *Nelumbo nucifera* extract): E: second treatment (P2: medium dose *Nelumbo nucifera* of extract); F: third treatment (P3: high dose *Nelumbo nucifera* of extract). Red arrow ( )

showing spermatogonium cells; green arrow ( ) showing spermatocytes; and blue arrow ( ) showing spermatids; (Hematoxylin Eosin: 400x magnification).

Based on the Table 3 and Figure 3 and 4, there is a significant difference between the positive control group (KP) induced by 2-ME with the drug group (KO) and treatment group (P1, P2 & P3), namely in the observation of the number of spermatogonia cells, in the observation of spermatocytes cells there was no significant difference between the negative control group (KN) with the drug group (KO) and the treatment group (P3) and in the calculation of the number of spermatid cells there was also no significant difference between the control group negative (KN) with treatment group (P3). Thus, it can be concluded that the administration of *Nelumbo nucifera* extract at a dose of 450 mg / kg bw is significantly able to repair damage to spermatogenic cells damaged by 2-ME induction. The results of this study were able to indicate that the antioxidant content contained in *Nelumbo nucifera* extract was able to restore the quality of spermatozoa damaged by induction of 2-*methoxyethanol* (2-ME).

Reproductive and developmental toxicity in animal models has been linked to 2-*Methoxyethanol* (2-ME). This substance has demonstrated significant toxicity specifically in male reproductive systems. Exposure to 2-ME in experimental animals causes a decrease in testicular weight, a decrease in the number of spermatogonia, spermatocytes and spermatid cells[21]. 2-ME compounds are not found naturally in the environment because their presence in nature is the result of industrial and factory activities. 2-ME enters the body of animals and humans through inhalation, oral and topical, which will then be oxidized by *Alcohol dehydrogenase to methoxyaldehyde* (MALD); and MALD is rapidly oxidized by *aldehyde dehydrogenase* to 2-*methoxyacetic acid* (2-MAA) which is a stable and highly toxic metabolite. Exposure to MAA in male mice can cause disruption of the reproductive system, especially in the testes. The main disorders occur in the process of spermatogenesis, germinal epithelial degeneration, infertility, abnormal spermatozoa morphology, can also cause apoptosis in spermatocytes[22].

The testes are the main reproductive organs of men and are responsible for producing spermatozoa and hormones. Spermatogenic cells that have not developed into spermatozoa cells have the potential to experience disruption due to *Reactive Oxygen Species* ROS[23]. The presence of free radicals due to increased ROS can be caused by exposure to chemicals that are toxic and are strong oxidants, one of which is 2-*Methoxyethano*l (2- ME). These free radicals will be very dangerous for several organs, one of which is the reproductive organs. ROS can also cause disruption in ATP production and apoptosis in cells[24,25]. Emerging data suggest that reactive oxygen species (ROS) can fulfill this role in the GnRH receptor signaling through activation of MAP kinase signaling cascades, control of negative feedback, and participation in the secretory process[26].

Exposure to free radical compounds 2-ME affect the work of the central nervous system by the release of *Gonadrotopin Releasing Hormone* (GnRH) by the hypothalamus in stimulating the release of *Luteinizing Hormone* (LH) and *Follicle Stimulating Hormone* (FSH). These hormones function as regulators of spermatogenesis activity in the testes, such as LH which functions to stimulate leydig cells in producing testosterone, and FSH which functions to stimulate sertoli cells in the process of spermatogenic cell formation[27,28]. There is a decrease in levels of the hormone due to the content of compounds in 2-ME cause impaired spermatogenic cell formation and impact on the quality of sperm produced. This corresponds to the function of the testicles as genital organs to produce sperm. Severe testicular loss (atrophy) can result in infertility[29]. Giving 2-ME to mice BALB-C affects spermatogenesis, which is a decrease in the number of spermatogenic cells, especially in spermatocyte cells I.[30] A decrease in the number of spermatocyte cells I is thought to involve cell apoptosis. 2-*methoxyethanol* also affects the anatomy of the seminiferous tubules of the testes, which are characterized by epithelial degradation and cause atrophy in the testes.

Various parts of the *Nelumbo nucifera* plant, such as leaves, roots, seeds, and flowers, contain several bioactive flavonoid molecules, including flavonols, flavones, flavan-3-ols, flavanones, and anthocyanins. Flavonols found in *Nelumbo nucifera* are *myricetin, quercetin, kaempferol,* and *isorhamnetin,* while the flavone molecules are *diosmetin, needletin, apigenin, luteolin*, and *chrysoeriol Nelumbo*[31]. *Quercetin* is also a polyhydroxy flavonoid that is widespread in plants, which has strong antioxidant and free radical exterminator abilities[32]. *Quercetin* is a flavonoid found in fruits and vegetables that has health benefits for example as, antimicrobial, anticancer, anti-inflammatory, antiviral and antioxidant[33].

The mechanism of action of flavonoid compounds can ward off free radicals, namely by reducing ROS. In the formation of ROS, oxygen will bind to free electrons that come out due to leaking electron chains[34]. This reaction between oxygen and free electrons is what produces ROS in mitochondria[35]. Antioxidants in flavonoids (*quercetin*) can donate hydrogen atoms so as to suppress the radical properties of free radicals. *Quercetin* will oxidize and bind to free radicals so that free radicals become more stable compounds[36]. In addition, *quercetin* can induce the antioxidant capacity of cells by activating the intracellular p38 MAPK pathway, increasing intracellular GSH levels and providing a source of hydrogen donation in counteracting free radical reactions[37].

The highest antioxidant activity test was found in White Lotus flower extract (*Nymphaea nouchali* L) with an IC50 value of 66.906 µg / mL, and the lowest was found in White Lotus leaf extract (*Nymphaea nouchali* L) with an IC50 value of 99.449 µg / mL[38]. *Quercetin* as a comparison standard has an IC50 value of 6.337 µg/mL with a "very strong" antioxidant activity category, while flower extract, flower stalk extract, leaf extract, and petiole extract have a "strong" antioxidant activity category. The maceration process of the extract uses a 70% ethanol solution. While in this study testing the antioxidant activity of *Nelumbo nucifera* extract has an IC50 value of 105.8 µg / mL, *Nelumbo nucifera* ethanol extract has moderate antioxidant activity. The maceration process uses a 96% ethanol solution. From the results of the study, there are different solutions used at the time of maceration and plant extracts used so that they produce different value results but both have moderate-strong antioxidant content that can delay or inhibit cell damage, especially through free radical antidote properties.

Therefore, compounds in the of *Nelumbo nucifera* show potential as candidates for anti-infertility drugs. In general, it can be concluded from this study that the optimal dose to prevent spermatocyte degeneration is the administration of *Nelumbo nucifera* extract at a high dose. However, more research in the field of phytopharmaceuticals is needed to consider *Nelumbo nucifera* as a potential remedy.

CONCLUSION

Antioxidant Potential of high-dose *Nelumbo nucifera* extract is able to repair spermatogenic cell damage after induced 2-ME. This shows the potential of *Nelumbo Nucifera* extract as a drug candidate against male infertility. But further research needs to be done on its potential in humans and to validate its use.

AUTHORS' CONTRIBUTIONS

Badriatul Musyarofah: Project administration, Conceptualization, in vivo examination, antioxidant measurement, analyze data; Angella Ananda Syaputra: Data curation, Writing- Original draft preparation. Amelia Kartika Reza; Yunita Ainul Kasanah: Methodology, Visualization, Investigation; Putri Ayu Ika Setiyowati: Resources, Supervision, Validation data, and Reviewing; Rofiatun Solekha: Supervision.

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DATA AVAILABILITY STATEMENT

There were no studies involving humans and animals as test objects in this research.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are the author's own and do not necessarily reflect the views or policies of the author's institution. The data is original research by the author and has not been published elsewhere.

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JTL 12 (1) MAY 2024
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Original Research

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GC-MS Analysis of phytochemical compounds from Javan olive leaves (Olea javanica) extract and bioactivity screening of antidiabetes through in silico approach

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Abstract: Diabetes is one of the most common metabolic diseases, ranking ninth in mortality worldwide. Although many effective hypoglycemic drugs are available for the treatment of diabetes, researchers are continually seeking more effective drugs with fewer side effects by focusing on various metabolic components such as enzymes, transporters, and receptors. The glucokinase (GK) enzyme, which is primarily found in the liver and pancreatic beta cells, is involved in maintaining blood glucose homeostasis. Therefore, this study was designed to determine the interaction between glucokinase and the compounds (ligands) from Javan olive leaf extract (*Olea javanica*) using GC-MS and in silico analysis. The method used in this study involved maceration with 96% ethanol to produce Javan olive leaf extract, followed by analysis via GC-MS using the Agilent 6980N Network GC System. Subsequently, in silico analysis was conducted using PyRx 0.9 software, the pkCSM website, BIOVIA Discovery Studio 2019, the RCSB PDB database, AutoDock Vina, the CABS-flex 2.0 web server, and Lipinski's rules. The results of the GC-MS analysis identified compounds such as 3 methylpentane, hexane, methylcyclopentan e, alpha-muurolene, (-)-calamenene, methyl 14-methylpentadecanoic acid, methyl ester linoleic acid, trans-squalene, and alpha-tocopherol. In silico analysis revealed that the molecules matching the target protein for diabetes treatment are native ligands exhibiting antidiabetic activity, as determined by molecular docking in this study.

Keywords: Diabetes, GC-MS, Glucokinase, In silico, *Olea javanica*

INTRODUCTION

The genus *Olea* comprises 12 species[1]. The family Oleaceae is commonly known as the olive family, with its members referred to as "olive" in English and "zaitoon" in Arabic. The health benefits of olive fruits and leaves have been widely researched concerning the treatment of respiratory diseases, urinary tract infections, and gastrointestinal disorders, while the oil is applied to the scalp to prevent bone loss and fractures[2]. In addition to traditional uses, the fruit and leaves have recently been reported to have antioxidant effects that could potentially benefit the skin[3].

Research suggests that leaf extracts from olive plants such as *Olea europaea* and *Olea ferruginea* have potential as antidiabetic agents[4,5]. One of the close relatives of the olive that grows in Indonesia is the Javan olive (*Olea javanica*). This plant is an endemic species of Indonesia that has not been extensively studied for its bioprospective potential. Oleuropein is a phenolic compound believed to have hypoglycemic, antihypertensive, antioxidant, anti-

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inflammatory, and cardioprotective properties, as well as supportive effects in obesity therapy.

In general, olive leaves have a rich content of phenols that are beneficial for human health[6]. Oleuropein is part of phenol compounds where oleuropein is thought to play a role as hypoglycemic, antihypertensive, antioxidant, anti-inflammatory, cardioprotective, and supportive effects in obesity therapy. Oleuropein has potential as a hypoglycemic effect with the ability to increase glucose uptake into cells, thus it can be used as an alternative therapy for diabetes to control blood sugar levels[7].

One of the research on diabetes control therapy is by using glucokinase control. Glucokinase (GK or hexokinase IV) as a glucose sensor plays an important role in glucose homeostasis[8]. In pancreatic β-cells, it regulates insulin secretion in response to circulating blood glucose levels. In the liver, it facilitates glycogen storage and post-meal glucose clearance from the bloodstream. Additionally, in pancreatic α-cells, it participates in glucose-dependent regulation of glucagon secretion.

One area of research in diabetes control therapy involves the regulation of glucokinase. Several studies have shown decreased levels of GK expression and activity in pancreatic cells in several animal models and humans with obesity and diabetes. Lu's research revealed that a high-fat diet can damage beta cells and induce diabetes[9]. This enzyme plays a crucial role in maintaining blood sugar balance in liver and pancreatic cells[11]. The use of separation techniques, such as liquid chromatography (LC) or gas chromatography (GC), prior to mass spectrometry (MS) detection, is common when analyzing complex plant-derived samples[12].

However, the chemical composition of Javan olive (*Olea javanica*) leaves remains largely unexplored. This study aims to identify phytochemicals through GC-MS analysis, quantify their abundance in Javan olive leaf extract, and assess their potential involvement in antidiabetic signaling pathways using an in silico approach.

MATERIAL AND METHOD

The materials used in this study included leaves from endemic Javan olive trees (*Olea javanica*) collected from wild populations in Mojokerto Regency, East Java. Additional materials used included 96% ethanol, filter paper, and aluminum foil. The laboratory equipment used in this study included an oven, analytical scales, beakers, dropper pipettes, a rotary evaporator, a vortex mixer, and a water bath.

Leaf Collection

Javan olive (*Olea javanica*) leaves were collected on July 1, 2023, from Mojokerto District, East Java, Indonesia (coordinates: -7.558369, +112.547966; altitude: 108.7 meters). The collected leaves were cleaned to remove dust and dirt, then dried in an oven at 60°C for 5 hours[14]. The dried leaves were then pulverized into a fine powder using a blender.

Olive Leaf Extraction

Olive leaf powder (250 grams) was homogenized using the maceration method by adding 96% ethanol (w/v) for 72 hours. The resulting macerate was filtered using filter paper and a rotary evaporator. The filtrate was then concentrated using a rotary vacuum evaporator and further processed with a water bath to produce pure olive leaf extract[15].

GC-MS Analysis

Javan olive leaf extract was dissolved in 6 mL of ethanol, vortexed for 2 minutes, and then sonicated for 15 minutes. The resulting solution was filtered through a 0.45 µm membrane filter, and a 1 µL aliquot was injected into the GC-MS instrument. GC-MS analysis was performed using an Agilent 6980N Network GC System equipped with an Agilent 5973 inert MSD detector and a J&W

Scientific HP-5MS column (0.25 mm × 30 m × 0.25 µm). The oven temperature was programmed to increase from 150°C to 230°C at a constant flow rate of 1 mL/min.

Mass spectra were obtained and compared with the Wiley spectral library (version 8.0), which contains more than 62,000 reference spectra[16]. Compounds were identified based on the similarity of their mass spectra, retention times, and molecular formulas. The relative abundance of each identified compound was determined by calculating its peak area as a percentage of the total peak area of all detected compounds[17].

In Silico Analysis

In silico docking simulations were performed to evaluate the binding interactions between secondary metabolites identified in Javan olive extract and target proteins.

Ligand Preparation

The 3D structures of the identified compounds (shown in Figure 2) were retrieved from public databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider (https://www.chemspider.com). For PubChem-derived ligands, conformational optimization was performed using the Open Babel 2.3.1 plug-in integrated within PyRx 0.9 software[18]. Ligands obtained from ChemSpider/1were downloaded in molar file format and converted to Canonical SMILES format using the CACTUS (Chemical Abstracting Computer Toolkit Service) website (https://cactus.nci.nih.gov/translate)[19]. Ligands obtained from ChemSpider were downloaded in mol file format and converted to Canonical SMILES format using the CACTUS (Chemical Abstracting Computer Toolkit Service) website (https://cactus.nci.nih.gov/translate)[19]. This optimization process enhances ligand flexibility and facilitates docking simulations[20].

Control Ligand Preparation

Control ligands are known modulators or activators of the target protein. In this study, glucokinase activators were used as control ligands.

Protein Preparation

The 3D structure of the target protein, human glucokinase (GCK), was retrieved from the Protein Data Bank (PDB) database (http://www.rcsb.org/pdb/home/home.do) using its specific identifier (ID). The protein structure was then processed using Biovia Discovery Studio 2019 software to remove any contaminant molecules[21].

Target Protein Modeling Analysis

Protein preparation primarily involved the removal of water molecules and any bound ligands to facilitate visualization, docking simulations, and potentially molecular dynamics simulations.

Specific Docking

Molecular docking simulations were conducted using AutoDock Vina, integrated within the PyRx 0.9 software platform. It is important to note that docking primarily focuses on predicting ligand interactions within the protein's active site. Binding affinity, a measure of the strength of protein-ligand interactions, is inversely correlated with stability; lower binding affinity values indicate more stable complexes[22].

Chemical Interactions

The resulting docking poses were visualized using Biovia Discovery Studio 2019 software to identify key interactions, including hydrogen bonds, hydrophobic interactions, electrostatic interactions, and unfavorable contacts.

The formation of multiple hydrogen bonds is often indicative of a stable protein-ligand complex, as these interactions contribute significantly to binding affinity[23].

Structural Visualizations

Both 3D and 2D visualizations were generated using Biovia Discovery Studio 2019 software. Initial 3D representations of the ligands and the target protein were created to provide a global perspective. Subsequently, the focus shifted to a 2D view of the protein-ligand binding interface for a detailed analysis of intermolecular interactions.

RESULTS AND DISCUSSION

GC-MS Analysis Result

Phytochemical analysis was conducted using gas chromatography-mass spectrometry (GC-MS). This technique enables the separation and identification of volatile compounds, including plant secondary metabolites. The resulting chromatogram visually represents the sample components, with each peak corresponding to a distinct compound[24]. The chromatogram of the ethanol extract from Javan olive leaves is presented in Figure 1, while the identified compounds and their relative abundances are summarized in Table 1.

Table 1. Chemical Components of GC-MS Test Results of Endemic Javan Olive Leaf Extract

Chemical Compound	RT (Retention Time)	Normalization%	Qual
3-methylpentane	1,81	2,71	91
Hexane	1,88	50,46	93
Methylcyclopentane	2,04	14,95	91
Alpha-muurolene	17,51	0,12	99
-(-calamenene)	17,81	0,12	91
Methyl 14-methylpentan decanoic acid	22,09	0,47	98
Methyl ester linoleic acid	23,80	0,89	99
Trans squalene	29,95	1,68	91
Alpha tocopherol	34,26	1,13	95

The chromatogram presented in Figure 1 illustrates the relative abundance of each identified chemical class as a percentage of the total chromatographic peak area, determined using five analytical methods. A total of 107 distinct compounds were detected in the chromatogram, with each peak corresponding to a unique chemical entity. However, the identification of certain compounds was hindered by low-quality scores (<90), indicating a lack of confidence in the spectral match. This discrepancy suggests that the sample's mass spectrum deviated significantly from the reference library spectrum, potentially due to factors such as background noise, ion source decomposition, weak signal intensity, or variations in collision energy. Consequently, this study provides an estimate of the overall percentage of unidentified compounds across the different analytical platforms. A substantial limitation of GC-MS phytochemical analysis is the necessity of authentic reference standards to precisely quantify and identify target analytes within intricate herbal matrices[25]. While GC-MS libraries offer a valuable resource for tentative compound identification based on spectral similarity, establishing definitive compound identity remains challenging due to potential spectral variations[26]. Known compounds identified in the GC-MS analysis included 3-methylpentane, hexane, methylcyclopentane, α-muurolene, (-

)-calamenene, methyl 14-methylpentanoate, methyl linoleate, trans-squalene, and α-tocopherol.

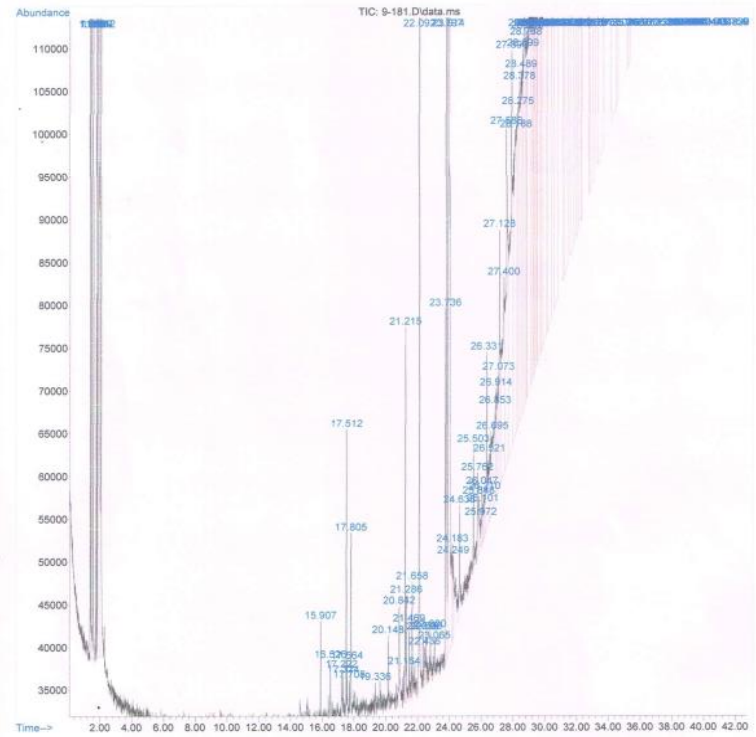


Figure 1. This is a figure GC-MS chromatogram of endemic javan olive leaf extract (*O. javanica*)

The chromatogram revealed nine distinct peaks, each subjected to mass spectrometric analysis. GC-MS analysis confirmed the presence of nine volatile compounds belonging to various chemical classes. In descending order of relative abundance, these classes were identified as esters (21.61-60.49%), alcohols (20.73-49.2%), hydrocarbons (3-38.88%), ketones (0.16-3.87%), acids (0.07-2.62%), and aldehydes (0.12-1.47%).

In Silico Results

GC-MS analysis identified several compounds[27] that adhered to Lipinski's rule of five, suggesting potential drug-like properties. These compounds were subjected to computational docking with the GCK protein, with the lowest binding energy pose selected for each. Binding energy is a predictor of protein-ligand complex stability, wherein lower values indicate stronger interactions[28]. Table 2 presents the docking results for the identified compounds: 3-methylpentane, hexane, methylcyclopentane, α-muurolene, (-)-calamenene, methyl 14-methylpentadecanoic acid, methyl linoleate, trans-squalene, and α-tocopherol, demonstrating their potential to form stable protein-ligand complexes.

Table 2. Results of molecular docking to determine binding affinity

Chemical Compound	Protein	Binding Affinity	RMSD
3-methylpentane	1,81	-4,1	0, 0
Hexane	1,88	-4,0	0, 0
Methylcyclopentane	2,04	-4,5	0, 0
Alpha-muurolene	17,51	**-7,9**	0, 0
-(-calamenene)	17,81	**-8,3**	0, 0
Methyl 14-methylpentan	22,09	-6,6	0, 0

decanoic acid			
Methyl ester linoleic acid	23,80	-6,4	0, 0
Trans squalene	29,95	**-7,9**	0, 0
Alpha tocopherol	34,26	-7,7	0, 0

Results of the Drug-likeness Property Assessment Based on Lipinski's Rule of Five

The drug-likeness properties of the identified compounds were evaluated using Lipinski's rule of five, which stipulates that drug-like molecules typically possess a molecular weight below 500 Da, a Log P value less than 5, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors[29]. As summarized in Table 3, α-muurolene and α-tocopherol from Javan olive leaf extract exceeded the Lipinski Log P threshold of 5. In contrast, 3-methylpentane, hexane, methylcyclopentane, methyl 14-methylpentadecanoic acid, methyl linoleate, and trans-squalene adhered to this criterion. Molecules with excessive hydrogen bond donors or acceptors tend to exhibit reduced chemical stability, potentially impacting their drug-like properties[30]. Non-compliance with any of these parameters can adversely affect a compound's absorption and bioavailabilit y[31].

Table 3. Results of the analysis of the properties of drug-like chemical compounds based on the Lipinski rule

Chemical Compound	MW (≤500 Da)	Log P (≤5)	HBD (≤5)	HBA (≤10)	MR(75-150)
3-methylpentane	86,18	3,52	0	0	30.96
Hexane	86,18	3,52	0	0	30,96
Methylcyclopentane	84,16	3,12	0	0	28,84
Alpha-muurolene	204,35	4,63	0	0	69,04
-(-calamenene)	202,34	5,45	0	0	68,07
Methyl 14-methylpentan decanoic acid	254,41	4,09	1	2	80,32
Methyl ester linoleic acid	294,47	4,09	0	2	93,78
Trans squalene	410,72	4,09	0	0	143,48
Alpha tocopherol	430,71	6,14	1	2	139,27

Protein Structure and Active Site

The target protein for in silico docking simulations was chosen based on its relevance to the study and the availability of a suitable structure. Ideally, the target protein should possess a known activatory ligand and a high-resolution structure deposited in the Protein Data Bank (PDB). In this study, human glucokinase (PDB ID: 6E0E) was selected as the target protein due to its established role in glucose metabolism and the availability of a complex structure with an activatory ligand (HKM) deposited in the PDB. Control compounds, defined as known activators of the target protein or its original ligand, were also identified based on information from scientific databases. The downloaded PDB file for human glucokinase contained information about the protein's three-dimensional (3D) structure, including its dimensions along the x, y, and z axes (23.6161 Å, 27.7023 Å, and 25.8563 Å, respectively) as reported in the reference source. The protein's active site residues were identified as Arg63, Pro66, Ile211, Val455, Met210, Tyr214, Ile159, Met235, Val62, and Val452. For visualization purposes, the 3D structure of the protein was rendered in a ribbon style, highlighting its secondary structure elements[33]. In this representation, red

indicates α-helices, light blue represents β-sheets, white represents loops, and green represents coils,as shown in Figure 2.

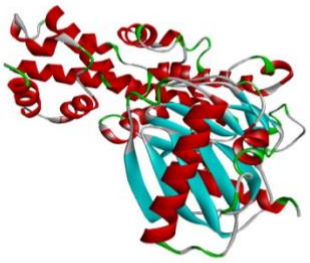
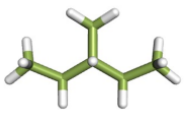
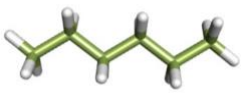
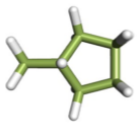
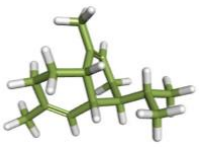
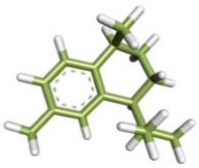
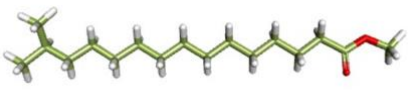
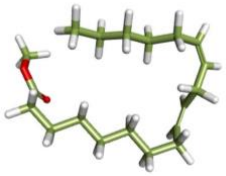
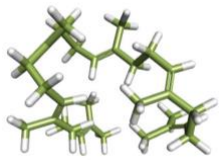
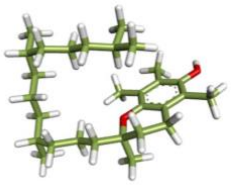


Figure 2. 3D structure of the target protein: Human glucokinase (insulin production activator)

The identified compounds within the *O. javanica* leaf extract are listed in Table 4, along with their corresponding Chemical Identifier (CID) and source database. The 3D structures of these compounds are depicted in stick representation in Figure 2, where carbon atoms are colored green, oxygen atoms red, and hydrogen atoms white. It is important to note that some compounds could not be identified or retrieved from the available databases.

Table 4. Phytochemical Compounds in PubChem: Those without HMDB and Those with HMDB

Chemical Compound	CID	Figure of Compound
3-methylpentane	7282	
Hexane	HMDB0029600	
Methylcyclopentane	7296	
Alpha-muurolene	12306047	
-(-calamenene)	HMDB0059910	
Methyl 14-methylpentadecanoic acid	HMDB0041422	

Methyl ester linoleic acid	HMDB0034381	
Trans squalene	HMDB0000256	
Alpha tocopherol	14985	

Molecular docking results of Javan olive compounds activating human glucokinase protein

Molecular docking simulations were performed to evaluate the binding interactions between the test compounds and human glucokinase. Binding affinity, a measure of the strength of the ligand-protein complex, was determined for each compound[34]. A lower binding energy value corresponds to a more stable complex, often indicative of increased activatory potency. The native ligand for glucokinase exhibited the most favorable binding affinity, serving as a benchmark for comparison. Similar to other known glucokinase activators, α-muurolene, trans-squalene, and (-)-calamenene displayed significant hydrophobic interactions with the protein. These compounds demonstrated potential as drug candidates based on their predicted binding modes, as visualized in Figure 3.

Table 6. List of Phytochemical Compounds in PubChem (without HMDB) and HMDB (with HMDB)

Ligand	Hydrogen Bonding	Hydrophobic Interactions	Other Bonds
Alpha-muurolene	-	Pro66, Pro66 (alkyl), Pro66, Tyr214, Tyr214 (pi-orbital)	-
-(-calamenene)	-	Trp99, Trp 99 (pi-pi stacked), Val101, Lys90, Val101 (alkyl), Trp99, Trp99, Trp99, Trp99 (pi-orbital)	-
Trans squalene	-	Pro66, Pro66, Pro66, Met210, Met235(alkyl), Tyr214, Tyr214, Tyr214 (pi-alkyl)	-
Native ligand	Arg63 (konvensional), Pro66 (C-H)	Ile211, Val455 (pi-sigma), Tyr214 (pi-pi T-shaped), Arg63, Ile159, Val455 (alkyl), Met236, Pro66, Val62, Val452 (pi-	Met210 (pi-sulfur)

alkyl)

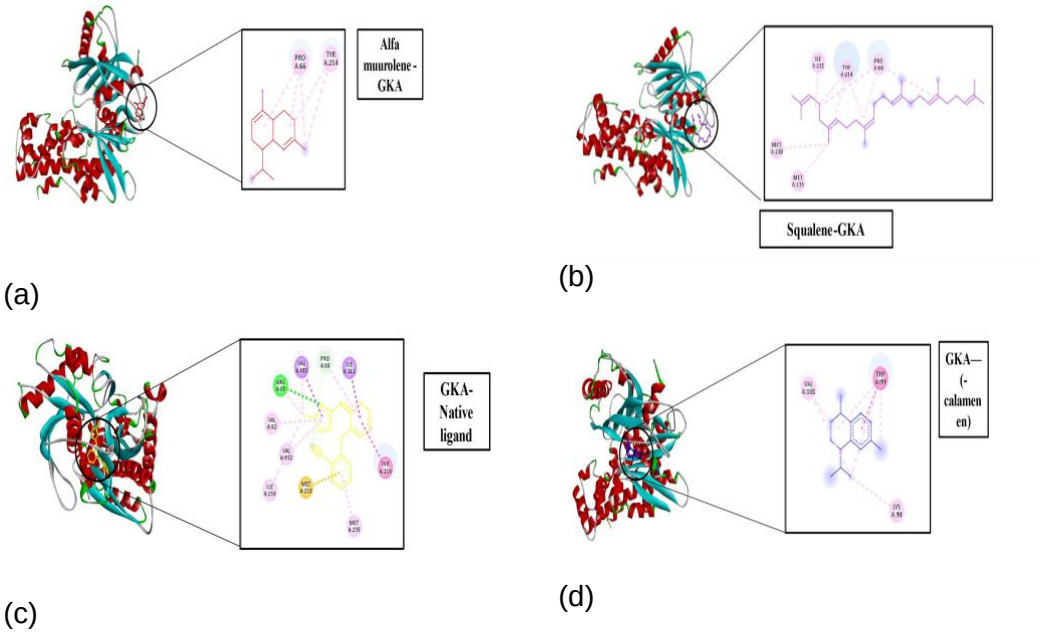


Figure 3. Binding mode visualization of (a) Interaction between human glucokinase protein and the chemical compound α-muurolene; (b) Interaction between human glucokinase protein and the chemical compound trans-squalene; (c) Interaction between human glucokinase protein and the native ligand; (d) Interaction between human glucokinase protein and the chemical compound (-)-calamenene.

Docking simulations between the identified compounds and human glucokinase revealed that α-muurolene, (-)-calamenene, and trans-squalene primarily formed hydrophobic interactions within the protein's active site. Figure 3a illustrates the interaction between α-muurolene and glucokinase, characterized by a binding energy of -7.9 kcal/mol and two π-alkyl interactions involving residues Pro66 and Tyr214. Similarly, trans-squalene exhibited a binding energy of -7.9 kcal/mol with a single π-alkyl interaction (Figure 3b). In contrast, the native ligand displayed a stronger binding affinity of -8.8 kcal/mol, forming four distinct interaction types: π-sigma, π-π T-shaped, alkyl, and hydrogen bonding (Figure 3c). Notably, the native ligand engaged with all active site residues. The (-)-calamenene ligand formed a binding complex with a binding energy of -8.3 kcal/mol, characterized by stacked π-π, alkyl, and π-orbital interactions with specific amino acid residues. Analysis of all docked compounds revealed a common motif of π-alkyl interactions involving Lys169 and Ile225. The presence of π-sigma interactions, including π-alkyl and π-sulfur subtypes, suggests potential charge transfer contributions to ligand binding[35]. These findings corroborate previous observations regarding the diverse chemical interactions of ligands compared to activators, emphasizing the importance of hydrogen bonding for protein stability and ligand affinity[36].

CONCLUSION

GC-MS analysis of Javan olive (*Olea javanica*) leaf extract identified a complex mixture of compounds, including 3-methylpentane, hexane, methylcyclopentane, α-muurolene, (-)-calamenene, methyl 14-methylpentadecanoate, methyl linoleate, trans-squalene, and α-tocopherol. In silico docking studies revealed that the compounds α-tocopherol, trans-squalene, (-)-calamenene, and the native ligand have properties that can activate

glucokinase to stimulate insulin production by binding to the target protein. However, the native ligand exhibits superior binding affinity due to its interaction with several amino acid residues within the active site of the protein. To substantiate these findings and advance the development of native ligand-based antidiabetic therapies, further in vitro, in vivo, and clinical studies are needed. These studies are expected to shed light on the therapeutic potential of *O. javanica* leaf extracts and pave the way for the discovery of new antidiabetic agents with different mechanisms of action.

AUTHORS' CONTRIBUTIONS

Lailatus Fitri: Con-septualization, software, and writing—preparation of the original draft; **Ayu Dewi Wulandari**: Con-septualization and formal analysis; **Tri sumaryono**: investigation and visualization; **Nur Fatimah Azzahra Haibaturrahma**: Methodology and data curation; **Bunga Nanda Agustina**: resources; **Muhammad Badrut Tamam**: validation, writing reviewing, editing, supervision, and project administration; acquisition of funding, Ministry of Education, Culture, Research and Technology.

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DATA AVAILABILITY STATEMENT

There were no studies involving humans and animals as test objects in this research.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are the author's own and do not necessarily reflect the views or policies of the author's institution. The data is original research by the author and has not been published elsewhere.

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